United States
Department of
Agriculture

Forest Service

Forest Pest
Management

Davis, CA

STUDY PLAN

ENVIRONMENTAL FATE OF *Bacillus thuringiensis* SPRAY APPLIED IN MOUNTAIN TERRAIN 1992 PHASE

Healthy Forests
Make A World
Of Difference

FPM 92-6
APRIL 1992

Pesticides used improperly can be injurious to human beings, animals, and plants. Follow the directions and heed all precautions on labels. Store pesticides in original containers under lock and key—out of the reach of children and animals—and away from food and feed.

Apply pesticides so that they do not endanger humans, livestock, crops, beneficial insects, fish, and wildlife. Do not apply pesticides where there is danger of drift when honey bees or other pollinating insects are visiting plants, or in ways that may contaminate water or leave illegal residues.

Avoid prolonged inhalation of pesticide sprays or dusts; wear protective clothing and equipment, if specified on the label.

If your hands become contaminated with a pesticide, do not eat or drink until you have washed. In case a pesticide is swallowed or gets in the eyes, follow the first aid treatment given on the label, and get prompt medical attention. If a pesticide is spilled on your skin or clothing, remove clothing immediately and wash skin thoroughly.

NOTE: Some States have restrictions on the use of certain pesticides. Check your State and local regulations. Also, because registrations of pesticides are under constant review by the U.S Environmental Protection Agency, consult your local forest pathologist, county agriculture agent, or State extension specialist to be sure the intended use is still registered.

FPM 92-6
APRIL 29, 1992

STUDY PLAN

Environmental Fate of *Bacillus thuringiensis* Spray Applied in Mountain Terrain-1992 Phase

Cooperators:

USDA Forest Service
  Intermountain Region - FPM
  Washington Office - FPM
Utah Department of Agriculture
U.S. Army Dugway Proving Ground
National Weather Service

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PREFACE

This plan covers field procedures to study the fate of Bacillus thuringiensis (Bt) in mountain terrain during May-June 1992. The study program will be conducted in Parley's Canyon and Lamb's Canyon, Salt Lake County, near the Mountain Dell Golf Course in conjunction with the 1992 Utah gypsy moth eradication project. A biological pesticide Bt will be applied by helicopter. The study is in follow-up to recommendations from Program WIND, (a U.S. Department of Agriculture-Forest Service (USDA-FS) and U.S. Army cooperative meteorological and computer model study); to recommendations from the USDA-FS national steering committees to further evaluate and technology transfer of computer models that predict the movement and deposition of sprays released from aircraft; and to the need for data on the environmental fate of Bt in the environment. The 1992 studies are a continuation of the 1991 study conducted in the same area. Scientists from the USDA-FS and U.S. Army, in cooperation with Utah State Department of Agriculture and National Weather Service will participate in one or more aspects of this study. The aerially Bt applied spray, to be sampled during these studies, will be sprayed by helicopter under operational conditions of the eradication project. Included in the 1992 program will be a study to investigate Bt soil levels in the 1991-1992 Parley's Canyon treatment area and a study to evaluate the penetration and deposition of spray in a maple canopy. A separate study or test plan will be prepared for the canopy study. No special spray treatment will be applied and no tracers will be added to the spray tank mix for the benefit of these studies. This study plan may be modified as needed and as agreed to by the cooperators. Results will be published in the open literature.

INTRODUCTION

Off-target movement of pesticides from forest spray operations has been a concern since aircraft were first used to spray trees (Neillie and Houser 1922). The concern primarily centers on potential environmental impact of pesticides on non-target species. Biological pesticides, such as Bacillus thuringiensis (Bt), are not exempt from this concern. Assessing potential environment impact of pesticides first requires quantitative data on the amount of pesticide that moves and deposits off the target site, followed by conducting environmental impact evaluations. Off-target movement is also a concern as it represents an inefficient use and economic waste of pesticides. For these reasons data are also needed to quantitate off-target movement that may lead to improving efficiency and efficacy of aerial spray operations. Predictions of the Forest Service Cramer-Barry-Grim (FSCBG) aerial spray model Teske (1990), a computer model that predicts travel and deposition of aerial sprays, has been compared favorable to several sets of observed field data and reported by GCA Corporation (1971); Boyle et al. (1975); Dumbauld et al. (1976); Dumbauld et al. (1977); Rafferty et al. (1987), Rafferty et al. (1988), Rafferty et al. (1989), and Teske et al., (1991).

Sampling off-target drift of pesticides in forests and in mountain terrain presents technical challenges. Researchers have had relatively few opportunities to obtain such data in mountains and few references are available in the literature, particularly biological aerosol studies (Sassaman, 1987). In situations where researchers have tried, results have been somewhat disappointing due to a variety of reasons including type of tracers and samplers used, sample contamination, and inadequate weather monitoring. Spray drift resulting from treatment of coniferous seed orchards has been reported by Barry et al. (1983); however the reported tests were conducted in relatively flat terrain. Rafferty et al. (1988) also reported deposition drift downwind to 2500 meters.

A study similar to the study proposed in this study plan was conducted in the immediate vicinity of the proposed study site in 1991 (Barry 1991). Results of this study clearly demonstrated that detectable amounts of Bt drifted at least 3,150 meters downwind from the treatment block. This is a continuation of the 1991 Study.

OBJECTIVE

The objective is subdivided into three tasks as listed below:

Task 1 - to quantitate off-site movement of Bt as measured by aerosol, impaction, deposition samplers, and Gambel oak foliage.

Task 2 - to compare FSCBG model predictions of air concentration (dosage and total dose) and deposition to observed data obtained from field samplers.

Task 3 - to investigate levels of Bt in mountain soils of Parley's Canyon.

SCOPE

The study and treatment sites are located in Salt Lake County, Utah, R2E, T1S, Sections 2,3,4,9,10, 11, 12, and 13. These sections, composed of public and private lands, are located in Parley's Canyon and Lamb's Canyon, along Interstate 80. The terrain is mountainous ranging in elevation from 5,000 to 8,000 feet MSL. At lower elevations the western and southern exposed slopes are partially covered at the lower elevations with Gambel oak. The site is ideally suited for these studies due to topography, channeling of drainage winds, and physical access.

The treatment site, designated as the Mill Creek Spray Block (SL-1), consists of 8,846 acres. Parley's and Lamb's Canyons are included in SL-1. The northeast half will be treated by a helicopter (Hughes 500D and/or Bell 206B-111) applying Bt in May-June 1992 to eradicate gypsy moth, a defoliator of oak and other deciduous trees. After the first spray is applied, treatment will be repeated two additional times at approximately five day intervals thus providing an opportunity for three replication trials. Off-site movement studies will be conducted only in the vicinity of Block SL-1. Spray moving down slope of the treatment area will be sampled by a variety of samplers positioned downwind to approximately 4.5 miles. Exact locations will be determined by GPS technology. Surface weather will be monitored by six stations and one tethersonde to measure upper air. Several organizations will cooperate in this study. A dry run will be conducted before May 15, 1992 to brief field crews, to practice field procedures, and to coordinate activities. A separate study plan will be prepared by DPG for a maple canopy study that will be conducted concurrently in the SL-1 treatment block..

METHODS - AEROSOL AND DEPOSIT SAMPLING

Application

The SL-1 Mill Creek Block will be treated operationally with Bt pesticide undiluted applied by helicopter at 0.5 gallons per acre. A total of 4,423 gallons will be applied; however only about half of the Bt will be applied in the portion of Lamb's Canyon that drains the airshed to be studied. Success of the study is dependent upon an organized drainage wind that results from nighttime cooling of slopes. To increase potential for a successful study the Treatment Supervisor's will be requested to:

1. Begin each of the 3 applications of SL-1 at first light when the pilots believe it is safe to fly and complete spraying before upslope winds begin.

2. Avoid spraying SL-1 if cloud cover precludes surface cooling. Drainage wind depends upon surface cooling that might not occur if clouds hold warm air near the surface.

3. Complete spraying in SL-1 before up-canyon winds develop. It would be ideal if Lamb's Canyon and the area near Parley's Canyon were treated at first light around 0530 hours.

4. Spray only the northeastern part of SL-1 Mill Creek Block, approximately 4,423 acres, on the days that the off-site movement study is conducted.

Field data collection requirements are listed in the paragraph Field Data Requirements. It is requested that the information listed be provided to the DPG Project Officer each day that Block SL-1 is treated.

Spray Material (Tank Mix)

The Bt to be applied (see attached pesticide label in Appendix) is a commercial formulation, Thuricide 48 LV produced by Sandoz Crop Protection Corporation, with a potency of 12,000 infectious units per milligram equivalent to 48 billion international units (BIU) per gallon. It will be applied undiluted at the rate of 0.5 gallons (24 BIU) per acre. No tracers or other additives will be added to the tank mix. The material has a relatively low rate of volatility. Data on the volatility of Thuricide 48LV should be available by late 1992. Wind tunnel testing of the atomization under conditions (atomizer, air speed, and application rate) approximating the anticipated operational conditions, is provided in Table 1.

Table 1 - Sampler activation/deactivation times in minutes based upon a wind speed of 2 meter per second, assumed mean transport wind.

Station	Distance[1.] Downwind meters	miles	Cloud[2.] Arrival	Sampler Activation	Cloud[3.] Passage	Sampler[4.] Deactivation
1-2	0	0	Z	Z-5		
3-4	500	.31	1.4	Z-5		
5-6	1100	.68	2.4	Z-5		
7-8	1750	1.09	1.09	Z-5		
9-10	2200	1.37	7.1	Z-5		
11-12	2800	1.74	10.1	Z-5		
13-14	3550	2.21	14.5	Z		
15-16	4150	2.58	17.5	Z		
17-18	4950	3.08	21.5	Z+5		
19-20	5950	3.40	25.7	Z+10		
21-22	6700	4.16	30.0	Z+15		
23-24	7500	4.66	34.2	Z+15		

Notes:

1. Distance downwind from center point of north boundary of SL-1 Mill Creek Block at Interstate 80, Parley's Canyon.

2. Z = Begin of spraying, all times relative to Z in minutes.

3. Cloud passage = cloud arrival + number of minutes required to complete morning spraying.

4. Sampler deactivation = cloud passage + 15 minutes.

5. Reynier schedule may differ.

Sampling

Approximate location of the sampling stations is shown in Figure 1. Off-site movement of Bt will be sampled downwind to approximately 4.5 miles for air concentration with spinning "U"-shaped brass Rotorod samplers spinning; for impaction with the "U"-shaped brass Rotorod[1] not spinning; and for deposition with Mylar sheets. A branch of Gambel oak leaves will be attached to each of the non-rotating Rotorod stakes. Reynier samplers with a turning disk holding an agar plate will be used to determine spray cloud arrival and departure times. Twenty-four sampling stations (12 pairs) will be positioned at 12 locations along a line that begins on the downwind edge of the SL-1 block and follows the drainage down-canyon from Lamb's and Parley's Canyon; and extending approximately 4.5 miles downwind.

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[1]*Rotorod^{R} is a registered trademark of Metronics Associates, Inc.

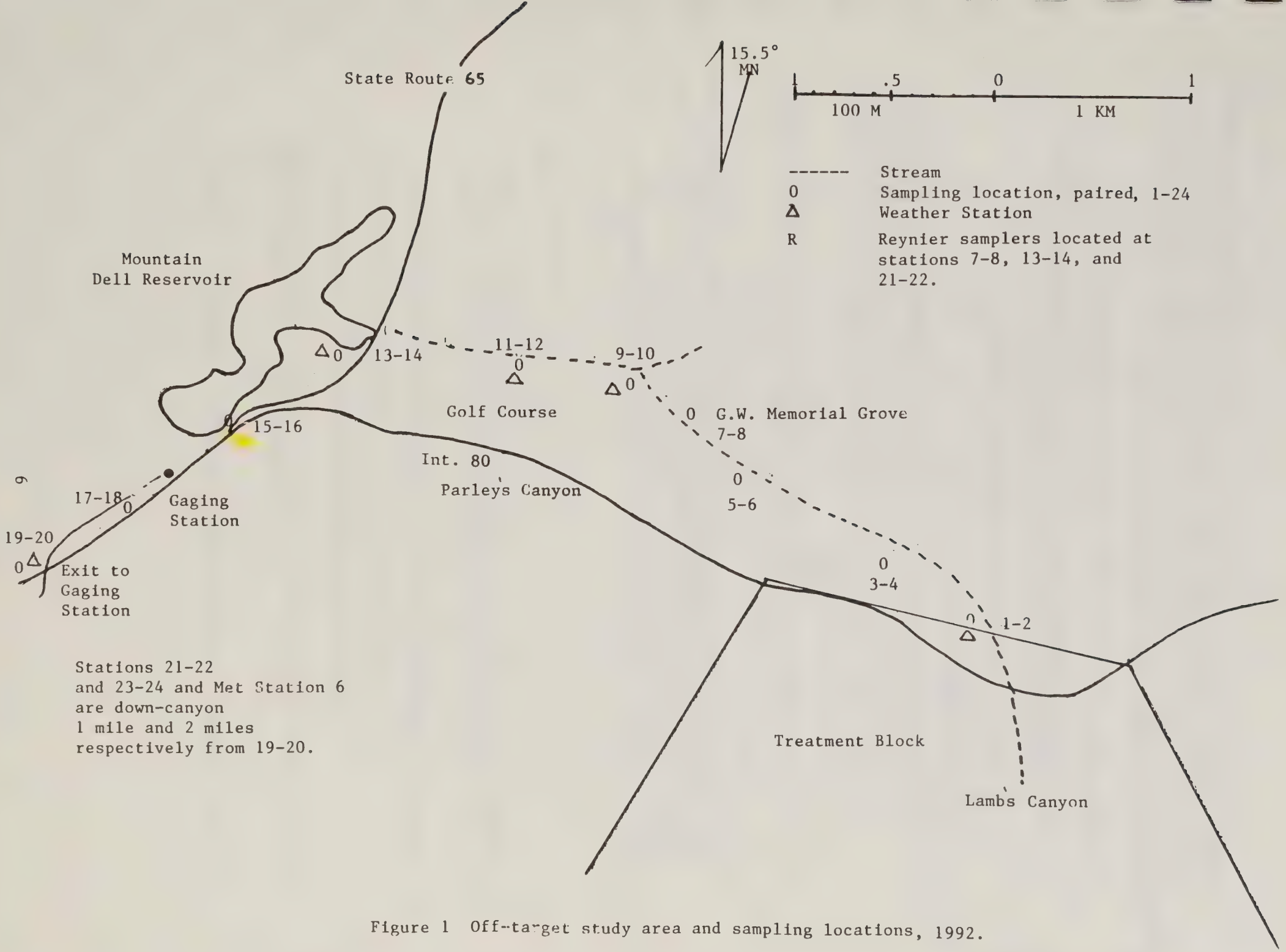


Figure 1 Off-target study area and sampling locations, 1992.

Samplers will be positioned and set-out the morning of treatment and picked-up after the spray operations and spray cloud passage is complete. The Test Officer will follow the sampling schedule (Table 1). The schedule assumes a 2 meter per second mean transport wind in activating and deactivating the samplers to insure sampling the entire spray cloud.

Sampling station (Figure 2) will consist of the following:

1. 2 each "U"-shaped brass Rotorods spinning clockwise at 2400 rpm, potentially sampling 120 liters per minute.

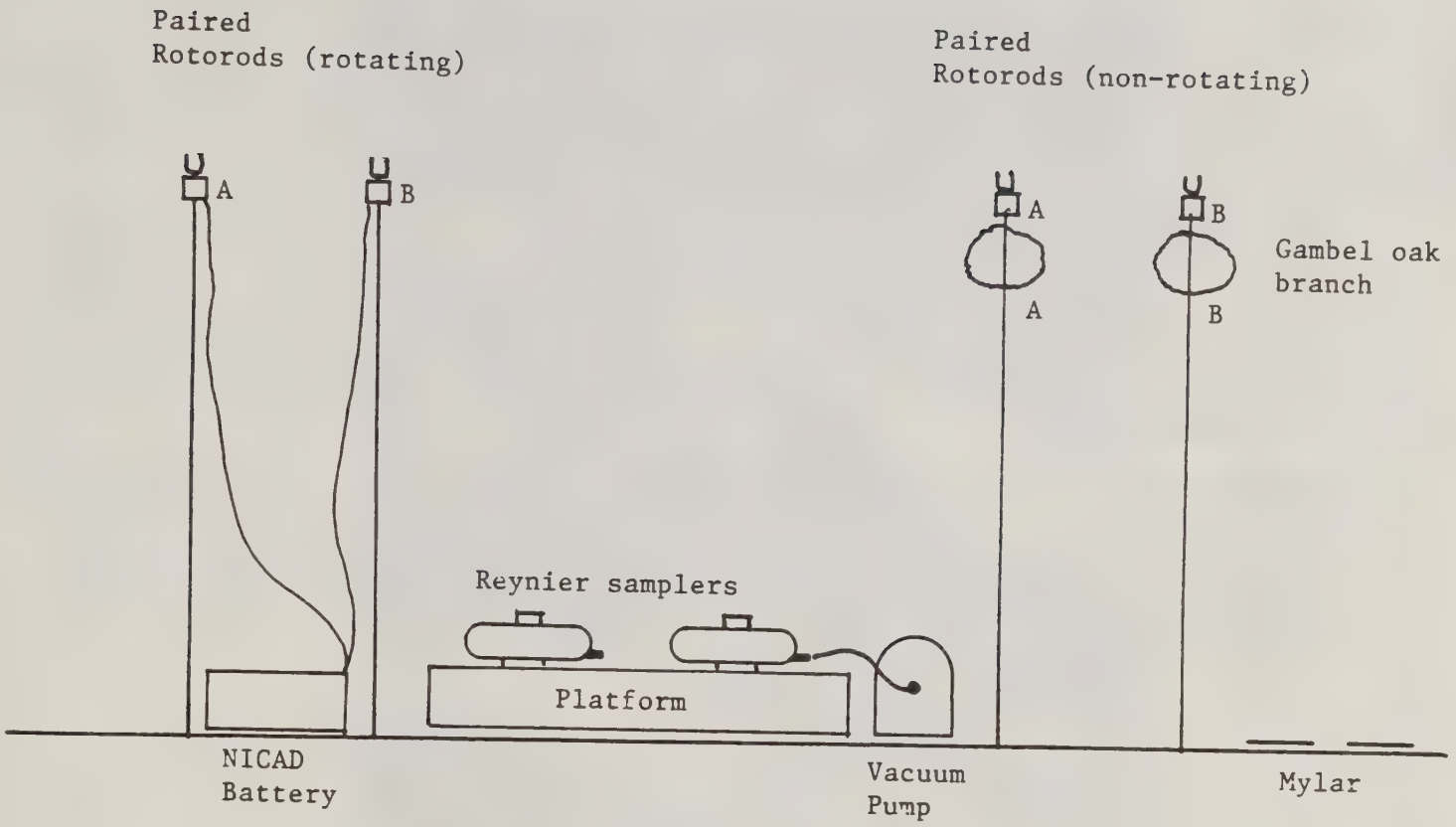
2. 2 each "U"-shaped brass Rotorods not spinning.

3. 2 each Mylar sheets on cardboard.

4. 2 branches approximately 8" in diameter of Gambel oak each containing several leaves collected morning of treatment. Five extra branches will be collected each morning as controls. These will be kept in paper bags, sealed, and protected from the spray and other possible sources of contamination.

5. 2 each Reynier samplers with 2-hour clocks will be placed at stations 7-8, 13-14, and 20-21. They will be operated at one CFM free flow and powered by a 110 V. AC generator..

The Rotorods will be elevated at 1.5 meters above the ground. The Mylar sheets will be attached to cardboard holders and in-turn these will be placed on boards at ground level (Figure 1). The board will insulate the samplers from moisture and help to reduce shielding from plants.



Notes:

- Samplers (Rotorods, Mylar, and Reynier) should be positioned perpendicular to axis of the drainage winds to avoid one sampler apparatus shielding the next.
- Non-rotating Rotorods arms will be oriented perpendicular to drainage wind.
- Reyniers only at stations 7, 8, 13, 14, 21, and 22.
- Nicad battery will be used to power the Rotorod motors.
- Generators used to operate the Reynier clocks and vacuum pump will be positioned downwind of samplers.

Figure 2 - Diagram of a sampling station with Reynier sampler, 1992.

The Test Officer will log and report times when each sampler is set out, picked up, activated, and deactivated. He also will record all malfunctions and missing samplers. This is critical to data analysis.

Rotorod[R] Sampler

The "U"-shaped brass Rotorod sampler (Figure 3) and technical bulletin in Appendix, developed by Metronics and currently produced by Ted Brown Associates, is a rotating arm impaction device capable of obtaining quantitative data of airborne particulates in the size range 10 to 100 micrometer diameter size (Brown 1976). At a nominal 2400 rpm which moves the collecting surfaces through the air and thus causes particles within the air intercepted by the collector rods to become impacted on the leading flat-surfaced edges of the rods. It samples 120 liters per minute when rotating at 2400 rpm with a 100% collection efficiency according to Edmonds (1972). The collecting surface of the "U"-shaped rod is 0.159 cm. Its basic components are a constant speed motor and aerodynamically designed collector rods which are rotated by a 12-volt motor. The Edmonds (1972) reference describes the sampler and provide instructions for its installation, operation and evaluation.

The spinning "U"-shaped brass polished Rotorod sampler will be used uncoated at each sampling station. The Rotorods will be used in pairs at each station, 2 rotating and 2 static. The rotating Rotorods will be connected to a 12-volt motor and powered by a 12-volt battery. The Rotorod assay will produce Bt colony forming units (CFU) per unit of time. This will yield dosage in CFU minutes per liter of air.

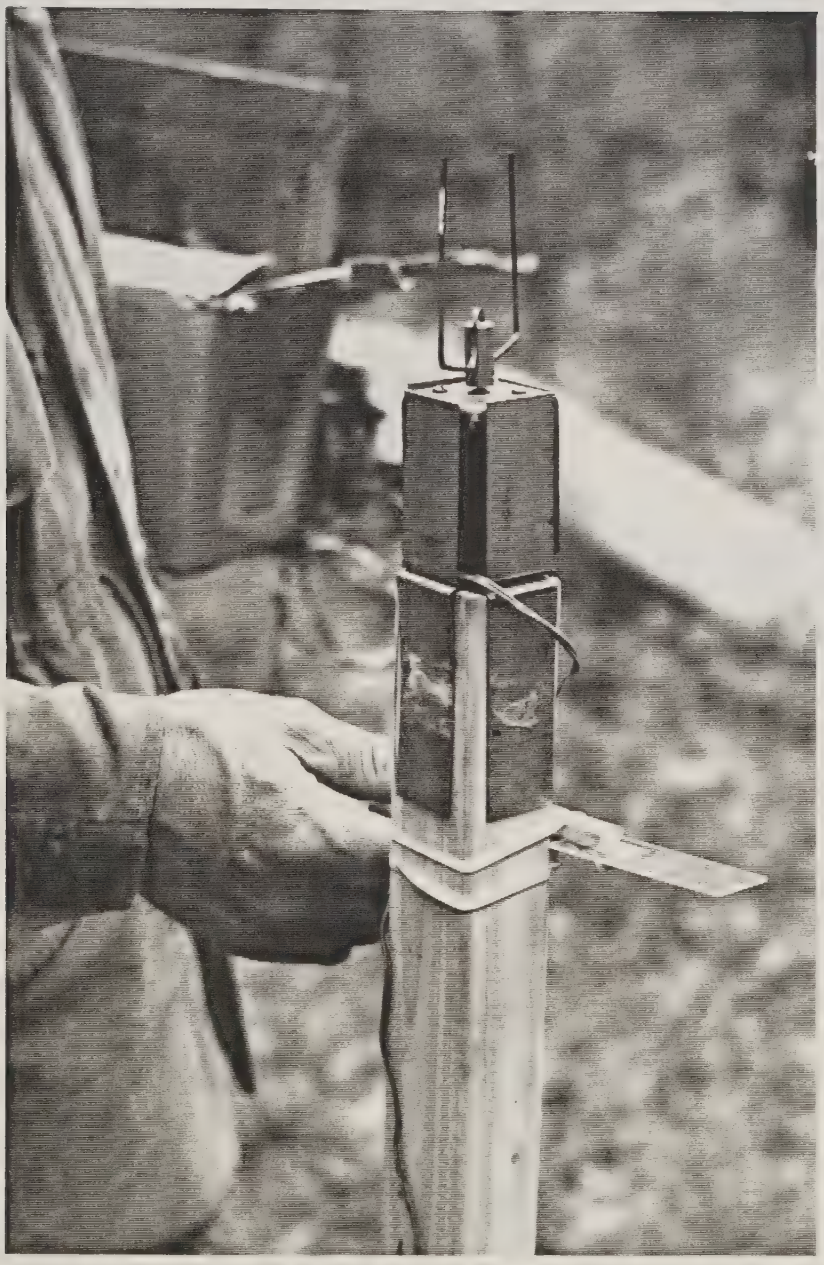


Figure 3. "U" shaped brass Rotorod sampler with 12-volt motor and holder for 2" x 2" stake.

Reynier Sampler

The Reynier sampler (Figure 4) is an aerosol sampler that collects bacterial aerosols directly on an agar plate. The plate rotates and provides colonies when aerosol could was at the location. Knowing when the sampler was activated one can pinpoint the aerosol cloud arrival and departure times. The Reynier operates on 110 - volts, AC and samples at one cubic foot of air per minute for two hours. Two Reyniers will be used at stations at 7, 8, 13, 14, 21, and 22 sequenced to provide a total of four hours of sampling. Instructions on preparation and use of Reyniers are in the Appendix.

Figure 4 - Reynier sampler with 2-hour clock.

Mylar Sheet

Mylar sheet, measuring 4 5/16 x 6 9/16 inches provided by the USDA-FS, will be positioned at ground level in duplicate. The Mylar will collect deposition resulting from gravitational settling.

Oak Leaves

A Gambel oak branch approximately 8" in diameter will be attached to each non-rotating Rotorod stake immediately below the Rotorod and on the upwind side of the stake.

Control Samples

Field control samples will be taken and standard DPG Life Sciences Laboratory operating procedures will be followed. A spinning Rotorod control will be operated for one hour prior to spraying at every even numbered sampling station. Controls will be packaged, marked and removed from the sampling area prior to spraying. A Mylar sheet control sampler also will be placed at every even numbered sampling station while setting up the station and picked up prior to commencement of spraying.

Quality Control

Applicable DPG standard operating procedures will be followed including quality control and use of control samples. A laboratory data report should also include results of laboratory control samples. Quality control includes both handling and exposure of control samplers and samples to detect Bt background, natural and accidental contamination of samples by Bt.

Review of the 1991 laboratory assay data suggest need for improved quality control in the field. Under supervision of the Field Test Officer and Crew Leader personnel should be especially aware of:

1. **Need** to pick-up all equipment needed for the trial;

2. **Need** to locate samplers to avoid golf course sprinkling;

3. **Need** to report all discrepancies e.g. contaminated and missing samples;

4. **Need** to follow the sampler marking system per this Study Plan; and

5. **Need** to follow sterile procedures in handling samples.

A liter of tank mix that represents the batch of Bt applied each day to SL-1 will be collected from the spray aircraft by the Utah State Liaison Officer and sent to the DPG laboratory for analyses. The sample should be placed in a plastic bottle, double packaged in plastic bags, and transported in an ice chest. The sample should be dated and Bt lot number placed on label.

Avoiding Contamination

The most serious threat to the integrity of the sampling is contamination of samples with Bt. Bt is a spore former that commonly occurs in the soil, thus there is potential for contamination from naturally occurring Bt. Being a spore former it is persistent as opposed to vegetative cells that are more susceptible to UV radiation and other degrading factors. Potential of contamination before the first spraying is minimal as Bt is not used at DPG. But once Bt is released contamination is a serious threat. After treatment personnel and the entire study area including foliage , soil, and other surfaces will be contaminated. Potential sources of sampler contamination include contaminated equipment, (sampling equipment, vehicles, containers) non-sterile samples, secondary aerosols (natural and man made), improper handling, packing, transportation of samplers, and contaminated crews (skin and clothing). Suggested procedures to reduce potential for contamination include:

1. Follow laboratory officers instructions on handling and transporting samplers.

2. Clean and sterilize Rotorod samplers. If Rotorods are accidentally contaminated by dropping on ground, touching with contaminated hands or gloves, etc. this should be reported to the Test Officer and marked on the sampler bag. Always use sterile gloves or ziploc bags between Rotorods and your hands.

3. Avoid creating dust and secondary aerosols near samplers.

4. Approach samplers on downwind side.

5. Insure that Mylar card holders are sterile.

6. Handle Mylar with sterile instruments (e.g. forceps) or sterile surgical gloves, remembering that once the exposed Mylar is touched the instrument or glove is contaminated.

7. Wear clean clothing daily.

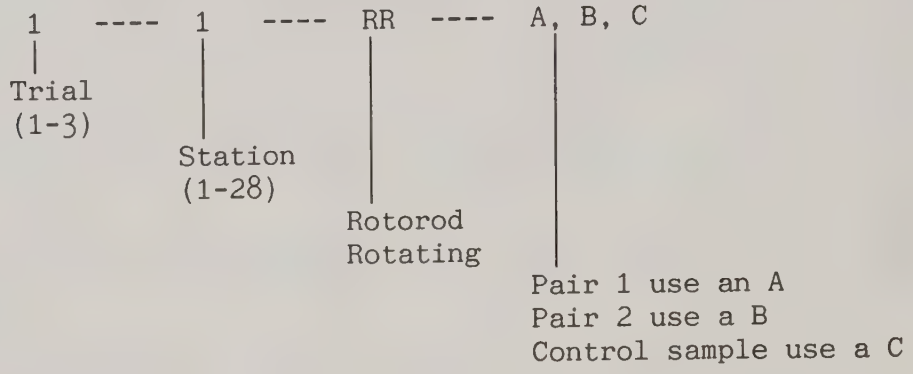
8. Keep vehicles and personnel upwind during trials - avoid the spray cloud.

9. Wash-down vehicles, racks, tote boxes, and other equipment after each spray day. This will help to reduce potential of contamination from secondary aerosols and cross contamination.

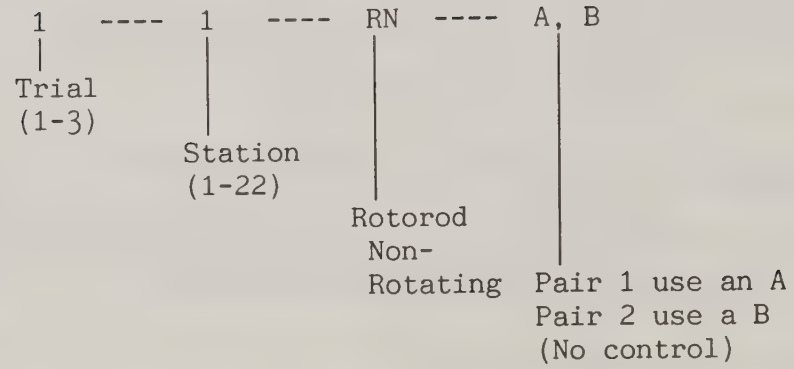
Sampler Marking

Sampler marking codes will be used throughout the study from the laboratory where the sampler is prepared through to the reporting of data. Codes will be marked on the outside of the Ziploc bags that contain Rotorod and Mylar samplers, and paper sacks that contain leaf samples. Errors in following the marking procedures will result in lost data.

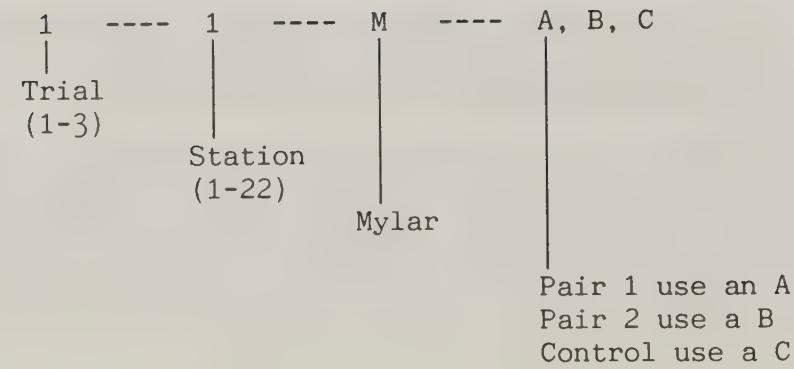
a. **Rotorod (Spinning)**



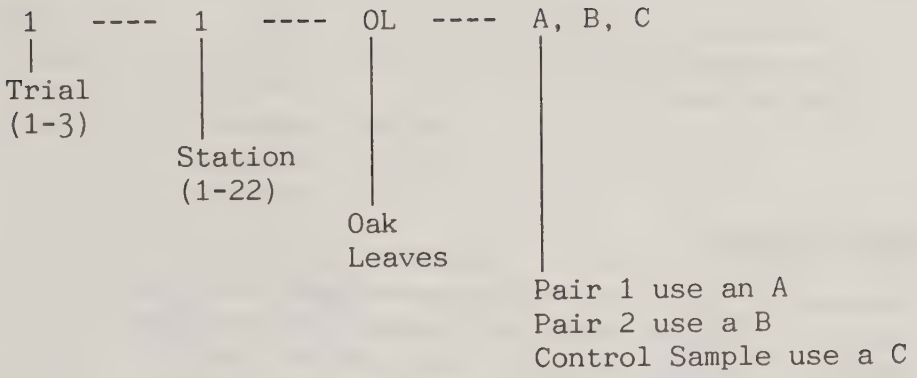
c. **Rotorod (non-spinning)**



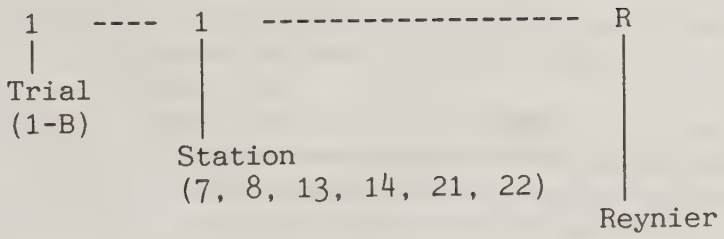
c. **Mylar Sheet**



d. **Oak Leaves**



e. **Reynier Sampler**



Sampling Equipment Requirements

There will be 12 paired sampling stations numbered 1-24 (Figure 2). The duplicates will be approximately at the same distance downwind but should be separated approximately 10 meters from each other where possible. DPG will provide all equipment and samplers except the Forest Service will provide Mylar.

Equipment (Per Trial)

	Required	Controls	Spare
NICAD Battery	6	--	6
Rotorod motors w/bracket	96	--	10
Stakes (2 x 2) for Rotorods	96	--	2
Mylar card holders	48	--	10
Generators, 110-V, AC	6	--	2

Samplers (Per Trial)

Rotorods spinning	48	12	12
Rotorods static	48	--	12
Mylar sheet	48	12	12
Reyniers	12	0	5

Laboratory Assay

Rotorods. Rotorods will be retrieved from the motor by covering the Rotorod with a Ziploc bag and sealing the zip without directly touching the rod. Labels will be placed on the outside of the Ziploc bag and not on the Rotorod. In the DPG laboratory the Bt will be extracted from the Rotorod and the Ziploc bag, and the diluent will be diluted and plated. Collecting fluid will be retained for additional assay as required.

Reyniers. Reyniers will be retrieved intact placed in the tote box and delivered to the laboratory as soon as possible.

Mylar Sheets. Mylar sheets will be retrieved in the field by sterile forceps and placed in sterile glass bottles or Ziploc bags. Bt will be extracted and plated-out in the same manner as the Rotorods at the DPG laboratory. Bt deposition could be high at those stations closest to the treatment block.

Oak Leaves. Oak leaves will be retrieved at the same time as the Rotorods. They will be placed in a paper sack and stapled shut, and the sack marked as per instructions.

Laboratory data and test officer's report should be provided to Study Director by August 1, 1992.

Weather Instrumentation and Measurements

Two or three solar powered weather EMCOT stations or comparable (Ekblad et al., 1990) and four each 2-meter weather stations will be used to collect wind speed and wind direction. Wind data will be collected at 2-second intervals to provide turbulence data by the FSCBG model. Dry and wet bulb temperatures and surface observations will be taken and recorded hourly at each of the six weather stations. Data collection will begin 15 minutes prior to beginning of spraying and continue until the last sampler is deactivated.

Field Data Requirements

Data requirements and person responsible for providing the data are listed below. These data will be needed for each of the three treatments of Block SL-1 Mill Creek.

1. Operational Treatment Data (R-4, John Anhold, Andy Knapp)

   Date
   Aircraft identification and description
   Pilot(s)
   Time block treatment began
   Time block treatment ended
   Total gallons applied
   Total acres treated
   Description of block treatment - specifically where the spray swaths were applied and when.
   Other remarks from pilot

2. Weather data from the 6 stations (DPG, Jim Rafferty) and (FS (MTDC), Harold Thistle))

   Wind speed
   Wind direction
   Temperature
   Relative humidity
   Cloud cover
   Barometric pressure (from National Weather Service, Salt Lake City airport)

3. Sampling (DPG, Test Officer)

   Time samplers set out
   Time samplers picked-up
   Time samplers were activated
   Time samplers were deactivated
   Time control samplers were activated, and deactivated, set out, and picked up
   Sampler malfunctions

4. Laboratory data (DPG, Gary Sutton)
   Total colony forming units (CFU) per each Rotorod and Mylar.
   Total CFU per gram dry weight of Gambel oak leaves.

5. Spray tank samples (Utah, Mark Quilter)

DATA ANALYSES

Data analyses, initially, will follow each task, and as appropriate, integrate results and analyses. Data from the laboratory and field controls will be analyzed and considered in the analyses and discussed in the report. Paired duplicate samplers have been included in the design to increase confidence as field sampling is recognized as being inherently highly variable.

A statistician will be contracted to analyze these data and to assist in preparing the data analyses section of the report and manuscript. Relationship of recoveries among different sampler types will be evaluated.

Task 1. Laboratory results will be compared and analyzed to determine a level of confidence for the types of samplers used and the resulting samples. Analyses will address questions to include: are recoveries within expectations and model predictions for given observed weather conditions and downwind distances; are recoveries relatively consistent; and what is the statistical relationship among the different sampler types? Deposition on the Mylar sheets and Gambel oak will be evaluated statistically by comparing duplicate recoveries as a function of downwind distance, and to FSCBG model predictions.

Task 2. Analyses under Task 2 will overlap that of Task 1 but focus on quantitative data and provide statements of statistical confidence in the quantitative recoveries.

Task 3. FSCBG model runs will be made after the field studies are completed. Input to the model will be the conditions existing during spraying supplemented by estimates of non-measured conditions e.g. height of the mixing layer and winds at spray release height. A statistical analyses of predictions among trials will be made along with significant differences that might be noted when input parameters are changed or modified during the FSCBG sensitivity analyses.

COORDINATION

Treatment Supervisor - John Anhold (801) 625-5292, FTS 586-5292
Responsible for overall conduct of the eradication program and providing manpower as requested.

Public Affairs Officer (PAO) - L.J. Western (801) 524-6207, FTS 588-6207
Responsible for all public affairs activities to include press releases, media contacts, public inquiries related to the program and studies, and coordination with Dick Whitaker, DPG PAO.

Study Director - Jack Barry (916) 758-4600, FTS 460-1715
Responsible for planning, coordination, documenting and reporting of the off-site spray movement study.

DPG Scientist - Bruce Grim (801) 831-3371
Responsible for all coordination administration and support with U.S. Army Dugway Proving Ground to include coordination between PAO and Dick Whitaker, Dugway public affairs officer and U.S. Army Aberdeen Proving Ground; and coordination with Dugway's Lockheed contract.

DPG Project Officer - Gary Sutton (801) 831-5638
Responsible for coordination with Test Officer, field crews, and Life Sciences Laboratory.

DPG Laboratory Officer - Lloyd Larsen (801) 831-5173
Responsible for preparation of samples, laboratory assay of samplers, quality control procedures, and reporting data.

Test Officer - Earl Davenport (801) 831-5247
Responsible for set-up operation, and pick-up of sampling equipment, inventory of equipment and samplers at pick-up and delivery, and quality control, and reporting on field operations.

Utah State Liaison Officer - Mark Quilter (801) 538-7190
Responsible for liaison, coordination among State and local jurisdictions, and study personnel.

Air Operations Officer - Andy Knapp (208) 364-4222
Responsible for providing data requested in paragraph 1 of Field Data Requirements.

SAFETY

Safety is everyone's responsibility both in practice and in reporting real and potential hazards. All personnel involved in this study will be familiar with and observe procedures outlined in the Operational Project Safety Plan and the DPG Safety Plan. Supervisors are responsible to insure that personnel read the Safety Plan and all personnel are responsible for safe work practices. The primary safety hazard is driving on Interstate 80, particularly access and egress; travel on unimproved roads; operating vehicles and equipment during early morning conditions; electrical hazards from generators; and lifting of equipment. Weather stations will be located to avoid electrical wires and vehicle and foot traffic. Any use of bucket trucks or "cherry pickers" will first require a site inspection and clearance by the safety officer. Guy wires and stakes will be marked with fluorescent engineering tape. Weather stations may be fenced if safety and security is deemed to be a problem. The material safety data sheet and pesticide label for Bt are in the Appendix.

REPORTING AND TECHNOLOGY TRANSFER

Results of this test and the analyses will be reported in a joint USDA Forest Service/U.S. Army report. If the field and laboratory procedures are successful, a manuscript will be prepared and submitted to the American Society of Agricultural Engineering, the Journal of Applied Meteorology, or other suitable professional publication for possible publication. In addition, a

paper will be offered for presentation to the summer or winter meeting of the American Society of Agricultural Engineers. Applicable results will be distributed to USDA-FS, Forest Pest Management offices and incorporated in training sessions directed at persons who develop environmental impact studies, and who plan and conduct aerial and ground spray operations.

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(3rd Draft 4/17/92)

STUDY PLAN

Detection, Quantification, and Persistence of *Bacillus thuringiensis* in Mountain Soil

by

John W. Barry[1]

Chi Li Liu[2]

Background and Introduction

The USDA Forest Service and its cooperators have a need for information on the fate of *Bacillus thuringiensis* Berliner var. *kurstaki* (HD-1 strain) (Bt) in the soil, air, water and on tree foliage. In 1990 and 1991 scientists from the Dugway Proving Ground (DPG) cooperated with FS scientists in conducting Bt fate studies in conjunction with the Utah gypsy moth eradication program. The FS and DPG have been partners for several years in developing and evaluating computer models that predict the deposition and dispersion of aerial sprays. An opportunity to continue the cooperative study is sustained by the FS Intermountain Region's 1992 gypsy moth eradication program and by DPG's willingness to provide technical support. The 1990 cooperative field work involved a canopy study along the Wasatch Front Range of Utah to measure the amount and location of Bt insecticide deposited on and captured by Gambel oak. The canopy work continued in 1991 at Mountain Dell in Parley's Canyon, Utah where a Bt off-target movement drift study was also conducted in conjunction with the 1991 program. The Bt insecticide FORAY 48B (Novo product) was applied in 1990-1991 by three applications at 0.5 gallons per acre per application. DPG scientists have drafted a report on the 1990-1991 canopy studies for publication spring 1992 and the FS is completing the off-site movement data analysis for reporting late spring 1992. The canopy study and off-site movement studies will continue in 1992 to address new questions and to confirm previous results. The 1992 data, supplemented with the 1990-1991 data, will be used to calibrate the canopy penetration code of the aerial spray model FSCBG and to evaluate the downwind drift codes of FSCBG.

The FS, Intermountain Region, Forest Pest Management Staff is supporting these studies to characterize the fate of Bt. The studies, that include use of qualitative and quantitative methods to characterize Bt in the soil, air, and on tree foliage, are being done to address questions on the fate and potential

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2. Chi Li Liu, Research Manager, Microbiology, Entotech, Inc., 1497 Drew Avenue, Davis, CA 95616, (916) 757-4740.

impact of Bt on non-target organisms when Bt is used as an insecticide to control gypsy moth. These questions have surfaced as concerns during the preparation of environmental impact analyses and during public meetings. Concerns have included a variety of general and specific questions to include: persistence of Bt insecticide in the soil; levels of natural Bt in the soil, air, and on tree foliage; contribution of Bt insecticide to natural Bt levels; levels of airborne Bt in the air during and after treatment; level of secondary Bt aerosols in areas at different periods after treatment; downwind distance that Bt insecticide spray might drift and quantification of such drift; and levels of Bt on foliage after treatment with Bt insecticides. Quebec, Canada has supported Bt environmental fate studies as reported by Dostie, Delisle, and Marotte (1989); Major and Rousseau (1987); and Delisle, Dostie, and Begin (1991). The latter studied the persistency of Bt in forest soils reporting persistence at low levels for two years after treatment. Persistence would be expected to vary depending upon Bt product formulation and environmental factors.

The gypsy moth eradication program is an ideal backdrop and economical approach for conducting the type of environmental fate studies described herein. For example there are no additional costs for application (insecticide and aircraft), and for project administration (project management, public affairs, and contracting services). Other economies result from efficient management of field personnel, vehicles, and other equipment. The backdrop also provides an opportunity to replicate the study as the eradication operation calls for three treatments of each block. From an intangible perspective such studies provide opportunities for research and operations personnel to work cooperatively in addressing needs of the resource manger. These factors promote effective technology transfer.

Scope

This study plan covers the purpose, objectives, and the procedures to be followed in conducting, analyzing, and reporting on the detection, identification, and persistence of Bt in mountain soil. The study will be conducted in Parley's Canyon, Utah along Interstate 80 between Mountain Dell Reservoir and Parley's Summit during May-June 1992. Cooperators in this study are the State of Utah Department of Agriculture, Entotech, Inc., Dugway Proving Ground, and USDA Forest Service. A series of 10 pairs of soil samples will be collected before commencement of the 1992 Utah gypsy moth treatment activities and again after the treatment with Entotech, Inc. assaying the soil samples for Bt. Laboratory results will be analyzed qualitatively and quantitatively for Bt. Further carry-on studies may be conducted subject to results of the pre and post treatment sampling.

Purpose

The purpose of this study is to collect data to supplement knowledge on the fate of Bt insecticides in mountain soils. This study will address the basic question on whether, and if so, how long might Bt insecticides persist and

contribute to the natural level of Bt in the mountain soils of Parley's Canyon, Utah. The Cooperators are interested in pursuing and reporting on questions identified in the Tasks described below under Objectives.

Objectives

The objectives are a number of questions subdivided into Tasks:

Task 1 - Does Bt occur naturally in the mountain soils of Parley's Canyon, and if detected what is the Bt spore count per gram of soil?

Task 2 - What are the levels of natural and/or other Bt in soil from sites in Parley's Canyon not previously treated in any year?

Task 3 - What are the Bt soil levels from 1992 pre-treatment levels and post-treatment levels 30 days, 6 months and 12 months after treatment?

Task 4 - Is there a correlation between Bt soil levels and Bt recoveries from deposition on Mylar samplers and air dosage Rotorod samplers?

Methods

In 1991 a total of 1,253 gallons of the Bt insecticide Foray 48B (Novo) was applied three times to 2,505 acres in Parley's Canyon for a total of 3,759 gallons. As part of a Bt off-target movement study, Bt deposition was measured at 6 sites downwind of the 2,080 acre Alexander Creek treatment block to 1,550 meters and airborne drift was measured downwind at 10 sites downwind to 3,150 meters. Bt was sampled with duplicate pairs of Mylar plates positioned approximately 107 cm above ground. Significant levels of Bt, expressed as colony forming units, were recovered from the Mylar. Other samplers were also used to quantitate the air concentration and dosage of Bt in the air. This background provides the basis and rationale for the methods approach.

Four series of soil samples will be taken with each series containing 10 duplicate samples numbered 1 through 10. The reason for deciding on 10 sampling sites relates to practicalities and economics, correspondence with spray deposition and air concentration sampling stations, and design used by Quebec researchers previously referenced. Each duplicate sample will be collected from the same site during the four serial collections. The study design critically depends upon control samples. Three sites will be controls taken from sites that were not treated since Utah gypsy moth spray commenced in 1989. The control sites will be analogous as possible to the other seven sites in solar exposure and soil type. Figure 1 is a diagram showing location of the 10 sites. Seven stations will correspond with four of the air sampling stations that will operate during the 1992 off-site movement study.

Each of the ten sampling sites will be marked with a metal stake and numbered 1 through 10. Two 12 inch squares, located in close proximity to the metal

stake, will be selected for soil collection. The duplicate sample will be a backup in the event of sample loss, contamination, or need for additional assessment. All soil samples will be collected using sterile procedures. Approximately 8 ounces of soil will be collected from the top 1 cm of soil depth and placed in a NASCO Whirl-Pak® sterile bag that measures 7.5 x 18.5 cm. At time of collection the bag will be marked to identify date and station number. Subsequent collections will be taken from squares immediately adjacent. Sample bags will be placed in a picnic cooler with ice and delivered to the assessing laboratory within 24 hours.

The first series of soil samples will be taken before commencement of spraying in Lamb's Canyon, on or before June 2, 1992.

The second series of soil samples will be collected 30 days following the third and final treatment in Lamb's Canyon. This period of time should allow for Bt that might be on vegetation to migrate to the organic litter in the grass root zone. It also will allow time for initial losses of Bt that result from ultra violet radiation and other environmental degradation processes.

The third series will be taken 6 months after treatment.

The fourth series of samples will be collected in 1993 twelve months after the third treatment in June 1992 subject to results of the third series of sampling.

Avoiding any possible source of or opportunity for contamination is paramount. Sources of contamination include people, clothing, containers, instruments, equipment, and vehicles. After the first treatment Bt spores will be abundant throughout the area; therefore extreme care must be exercised in collecting the soil samples to avoid contamination from the above listed sources directly or by secondary aerosol. In addition to using a sterile bag for soil collecting at each location a sterile metal spoon, provided with each bag, will be used to collect the soil sample, and a fresh pair of sterile surgical gloves will be used at each collecting site. Good laboratory practices will be followed in conduct of this study.

Data Analyses

Results of the laboratory assessment will be analyzed qualitatively and quantitatively consistent with the objectives/tasks of the study. Statistical differences will be explored to describe recoveries of Bt from control samples and as a function of time. Particular emphasis will be placed on determining whether Bt occurs naturally in the study area, at what levels it might occur; and to what extent, if any, did the Bt insecticide treatments in 1991 and 1992 contribute to the Bt soil burden. If Bt is detected in the 1993 samples, the Bt persistency study may continue into 1994 and a regression analyses will be performed to demonstrate and describe environmental degradation of the Bt.

Schedule of Events

	DATE	Event
1.	May 1992	Locate and mark location of the 10 soil sampling stations.
2.	June 2, 1992	Collect **First Series** (pre-treatment) soil samples.
3.	(Thirty days after final 1992 treatment)	Collect **Second Series** (post-treatment) soil samples.
4.	December 2, 1992	Collect **Third Series** (post-treatment) soil samples.
5.	June 2, 1993*	Collect **Fourth Series** (post-treatment) soil samples.

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* Event 5 is dependent upon results of event 4.

Coordination/Responsibilities

1. USDA Forest Service

Treatment Supervisor - John Anhold
Ogden Field Office
4746 South 1900 East
Ogden, UT 84403
(801) 476-9723
FTS 321-9720
FAX (801) 625-5127 (Regional Office)

Responsible for over all management of gypsy moth eradication program in Utah.

Study Coordinator - John W. Barry
Forest Pest Management
2121C Second Street, Suite 102
Davis, CA 95616
(916) 758-4600
FTS 460-1715
FAX (916) 757-8381

Responsible for planning, coordinating, and reporting results of the Bt soil study.

2. Entotech, Inc.

Research Microbiologist - Chi Li Liu
1497 Drew Avenue
Davis, CA 95616-4880
(916) 757-4740
FAX (916) 757-4789

Responsible for providing soil shipping permits, collection bags, and conducting microbiological assay of soil samples.

3. U.S. Army Dugway Proving Ground

Microbiologist - Lloyd Larsen
Attn: STEDP-MT-L (L. Larsen)
Dugway, UT 84022
(801) 831-5173
FAX (801) 831-5716

Responsible for collection of soil samples and delivery of soil samples to the assessing laboratory.

Project Officer - Bruce Grim
Attn: STEDP-SD-TA (B. Grim)
Dugway, UT 84022
(801) 831-3371
FAX (801) 831-2397

Responsible for coordination of Dugway activities/support of the Bt soil study.

Technology Transfer

Results of this study will be submitted for publication by the cooperators with joint authorship. Prior to manuscript submission the results will be reported in a FS report and made available for reference in environmental documents and at public meetings.

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4. Major, Lue and Ghislain Rousseau. 1987. Concentrations D'Insecticides Dans L'Air Aux Sites de Travail Lors des Pulverisations Aeriennes Contre la Tordeuse des Bourgeons de L'Epinette, en 1985. Services des Etudes Environmentales; Direction de la Conservation Ministere de L'Energie et des Ressources Gouvernement du Quebec.

THURICIDE® 48 LV

AQUEOUS CONCENTRATE FOR AERIAL OR GROUND APPLICATION FOR CONTROL OF ORNAMENTAL, SHADE TREE, AND FOREST PESTS

ACTIVE INGREDIENT:

Bacillus thuringiensis, subspecies kurstaki, potency 12,000 International Units (at least 18 million viable spores) per milligram*...	2.4%
INERT INGREDIENTS:	97.6%
TOTAL	100.0%

*Equivalent to 12.0 billion International Units per quart.

KEEP OUT OF REACH OF CHILDREN

CAUTION

EPA Reg. No. 55947-74
EPA Est. No. 55947-CA-2
MADE IN U.S.A.

Net Contents: 5 Gal. or 53 Gal.
Lot No.:

THURICIDE® is a Registered Trademark of Sandoz Ltd.

SANDOZ CROP PROTECTION CORPORATION
Des Plaines, Illinois 60018

December 1991

PRECAUTIONARY STATEMENTS

HAZARDS TO HUMANS

Avoid inhalation or contact with eyes or open wounds.

ENVIRONMENTAL HAZARDS

Do not contaminate water when disposing of equipment washwaters.

DIRECTIONS FOR USE

It is a violation of Federal Law to use this product in a manner inconsistent with its labeling.

Suspension must be shaken or stirred before use. Pour the recommended amount of THURICIDE® 48LV into the required volume of water in the spray tank. Agitate as necessary to maintain suspension. Do not allow diluted sprays to remain in the tank for more than 72 hours. THURICIDE® 48LV is formulated to provide desirable coverage and stickability on leaf surfaces. Additional adjuvants, spreaders, or stickers may be added but are not essential.

Do not apply this product through any type of irrigation system.

GROUND APPLICATION

Use adequate water to obtain good foliar coverage. Wet foliage but do not allow excessive run-off. Apply the recommended per acre rates of THURICIDE® 48LV with the following suggested amounts of water:

100 gallons with high-gallonage hydraulic sprayers
10 gallons with mist blower

AERIAL APPLICATION

Apply THURICIDE® 48LV at recommended rates by air either alone or as a spray mix diluted with water. Spray volumes of 32-128 ounces per acre are recommended. Best results are expected when THURICIDE® 48LV is applied to dry foliage with a calibrated aircraft capable of obtaining droplet sizes below 300 microns and preferably in the range of 50-150 microns.

RECOMMENDATIONS

Thorough coverage is essential when using THURICIDE® 48LV. Use the lower rate for light to moderate infestations. Use the higher rates against heavier worm infestations.

Pest	Ounces Per Acre	Dosage BIU Per Acre	Directions For Use
Spring Cankerworm	11- 44	4-16	Apply when leaf expansion reaches 40-50% as infestation warrants. If eggs hatch over a long period of time or if reinfestation occurs, respray about 14 days after the first application.
Fall Cankerworm	11- 44	4-16	
Elm Spanworm	11- 44	4-16	
Tent Caterpillar	11- 44	4-16	
Gypsy Moth	22-106	8-40	
Spruce Budworm	16-106	6-40	Apply when most larvae are 3rd-4th instar. Also consider the opening of the bud cap to ensure foliage exposure.
Douglas Fir Tussock Moth	11- 44	4-16	Apply after eggs have hatched and early instar larvae are feeding on exposed foliage.
Jack Pine Budworm	11- 44	4-16	
Bagworm	11- 44	4-16	
California Oak Moth	11- 44	4-16	
Western Tussock Moth	11- 44	4-16	
Fruit Tree Leafroller	11- 44	4-16	
Mimosa Webworm	11- 44	4-16	
Redhumped Caterpillar	5- 32	2-12	
Fall Webworm	5- 22	2- 8	
Pine Butterfly	16- 64	6-24	

STORAGE

Store in a cool place. Activity may be impaired by storage at temperatures above 90° F. Do not contaminate water, food, or feed by storage or disposal.

DISPOSAL

PESTICIDE

Wastes resulting from this product may be disposed on on-site or at an approved waste disposal facility.

CONTAINER (PLASTIC)

Triple rinse (or equivalent). Then offer for recycling or reconditioning, or puncture and dispose of in a sanitary landfill, or by incineration, or if allowed by State and local authorities, by burning. If burned, stay out of smoke. Reuse of thoroughly cleaned container is allowable.

CONTAINER (METAL)

Triple rinse (or equivalent). Then offer for recycling or reconditioning, or puncture and dispose of in a sanitary landfill, or by other procedures approved by State and local authorities. Reuse of thoroughly cleaned container is allowable.

LIMITATION OF WARRANTY AND LIMITATION OF LIABILITY

NOTICE: Read this Limitation of Warranty and Limitation of Liability before buying or using this product. If the terms are not acceptable, return the product at once, unopened, and the purchase price will be refunded.

It is impossible to eliminate all risks inherently associated with the use of this product. Crop injury, ineffectiveness, or other unintended consequences may result because of such factors as weather conditions, presence of other materials, or the manner of use or application, all of which are beyond the control of Sandoz or seller. All such risks shall be assumed by buyer or user.

Sandoz warrants that this product conforms to the chemical description on the label and is reasonably fit for the purposes stated in the Directions for Use, under normal use conditions, subject to the risks described above. **SANDOZ MAKES NO OTHER EXPRESS OR IMPLIED WARRANTY OF FITNESS OR OF MERCHANTABLILITY OR ANY OTHER EXPRESS OR IMPLIED WARRANTY.**

In no event shall Sandoz or seller be liable for any incidental, consequential or special damages resulting from the use or handling of this product. **THE EXCLUSIVE REMEDY OF THE USER OR BUYER, AND THE EXCLUSIVE LIABLILITY OF SANDOZ OR SELLER FOR ANY AND ALL CLAIMS, LOSSES, INJURIES OR DAMAGES (INCLUDING CLAIMS BASED ON BREACH OF WARRANTY, CONTRACT, NEGLIGENCE, TORT, STRICT LIABILITY OR OTHERWISE) RESULTING FROM THE USE OR HANDLING OF THIS PRODUCT, SHALL BE THE RETURN OF THE PURCHASE PRICE OF THE PRODUCT OR, AT THE ELECTION OF SANDOZ OR SELLER, THE REPLACEMENT OF THE PRODUCT.**

Sandoz and seller offer this product, and buyer and user accept it, subject to the foregoing limitations of warranty and limitation of liability, which may not be modified by any oral or written agreement.

SANDOZCROP PROTECTION

Date Issued: April 1, 1987
Supersedes: January 5, 1987

MATERIAL SAFETY DATA SHEET

THURICIDE® 48LV BIOLOGICAL INSECTICIDE

Product/Material: THURICIDE® 48LV BIOLOGICAL INSECTICIDE
Manufacturer: SANDOZ CROP PROTECTION CORPORATION
Address: 1300 East Touhy Avenue, Des Plaines, Illinois 60018
Emergency Phone: 312/699-1616

I. PRODUCT INFORMATION

Trade Name	THURICIDE® 48LV
Chemical Family	Biological Insecticide-Microbial. *Bacillus thuringiensis* Berliner Var. *Kurstaki*
EPA Reg. No.	55947-74
DOT Hazard Class	Non-hazardous

II. HEALTH/SAFETY ALERT

Active ingredient has not shown mammalian toxicity.

III. FIRST AID PROCEDURES

CAUTION **AVOID INHALATION OR CONTACT WITH EYES OR OPEN WOUNDS.**

IV. FIRE AND EXPLOSION INFORMATION

Flammable Limits	NA
Flash Point	NA
Autoignition Temperature	NA
Extinguishing Media	NA
Fire Fighting Protection	NA
Unusual Fire Hazards	NA

V. SPILL CONTROL AND CLEANUP

Steps to be taken Release or Spill Procedures Soak up with absorbent material.

VI. PRODUCT/WASTE DISPOSAL

Waste Disposal Procedures Waste resulting from this product may be disposed of on site or at an approved waste disposal facility.

VII. SPECIAL PRECAUTIONS

HANDLING AND STORAGE PRECAUTIONS

Precautions Store in a cool place. Do not contaminate water, food or feed by storage or disposal.

Personal Protection Use ordinary personal hygiene practice.

Shipping Name Thuricide 48LV

DOT Labeling Required

(X) None Required
( ) Oxidizer
( ) Poison
( ) Flammable
( ) Corrosive
( ) _____

VIII. HEALTH HAZARD INFORMATION

SIGNS AND SYMPTOMS OF ACUTE OVEREXPOSURE

Effects of Overexposure There have been no reports of adverse effects to humans resulting from overexposure to Bacillus thuringiensis. The active ingredient *Bacillus thuringiensis* has shown no mammalian toxicity. It is practically non-toxic, non-allergenic and non-pathogenic to mammals.

IX. PRODUCT INFORMATION—HAZARDOUS INGREDIENTS

REACTIVITY

Stability	(X) Stable	( ) Unstable
Hazardous Polymerization	( ) May Occur	(X) Will Not Occur
Incompatible Materials	NA	
Hazardous Decomposition Products	NA	
Conditions to Avoid	Avoid exposure to sunlight or heat to maintain activity.	

X. PHYSICAL AND CHEMICAL INFORMATION

Appearance and Odor	Pea green liquid, fermentation odor.
Boiling Point	NA
Melting Point	NA
Vapor Pressure	NA
Vapor Density	NA
Specific Gravity	1.14
Solubility	Suspendable in water
Evaporation Rate	NA
Weight/Gallon	9 lbs./gal.
pH	4.5 +/− 0.1
% Volatile	NA

The information presented herein, while not guaranteed, was prepared by technically knowledgeable personnel and to the best of our knowledge is true and accurate. It is not intended to be all inclusive and the manner and conditions of use and handling may involve other or additional considerations.

Information on this form is furnished solely for the purpose of compliance with the Occupational Safety and Health Act of 1970 and shall not be used for any other purpose. Use or dissemination of all or any part of this information for any other purpose or purposes is illegal.

COLLECTION EFFICIENCY OF ROTOROD SAMPLERS FOR SAMPLING FUNGUS SPORES IN THE ATMOSPHERE

Robert L. Edmonds [1]

Abstract

In the sampling of fungus spores in the atmosphere, the collection efficiency, and thus the accuracy of the samplers for obtaining quantitative data, has rarely been considered for the particular fungus spore being sampled. This paper is designed to make potential users of Rotorod impaction aerosol samplers aware of the importance of considering sampling efficiency. A method for calculating efficiency is given.

Adequate study of the dispersion of fungus spores requires accurate sampling of the atmosphere. In many investigations the collection efficiency, and thus the accuracy of samplers, has not been considered for the particular type of fungus spore being sampled.

Suction devices and rotating arm impactors are the most common types of instruments used in the collection of fungus spores. Because of the large size range of fungus spores (from a few microns (μ) to 100μ), however, there is no one instrument yet developed that is capable of sampling the whole range with equal efficiency.

This paper proposes to indicate to users of rotating arm impaction samplers the importance of considering collection efficiency in sampling; to discuss theoretical aspects in determination of efficiencies; to provide an equation for determination of sampling efficiencies for various sizes of fungus spores; to demonstrate how collecting surfaces can be modified to in-

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crease sampling efficiency; and to discuss the importance of the selection of a suitable sticky material for the leading edge of the sampler. The "Rotorod sampler" is used as an example of this type of device. It is commercially available and widely used.

The "Rotorod sampler" in the form developed and marketed by Metronics Associates, Inc. of Palo Alto, California (10) has been used for collecting fungus spores in the atmosphere by many workers including Asai (1), Froyd (5), Barksdale (2), Skilling (9), and Edmonds (4). It employs the process of inertial impaction with spores, and so forth, being impacted on a whirling arm.

Advantages of this sampler are low cost, simplicity, light weight, large sampling volume, suitability for experiments employing large numbers of simultaneous samplers, battery operation for remote locations, and the collection efficiency is not affected by wind speed up to 6.2 kph. The chief disadvantage is that collection efficiency is sharply dependent on spore size and density, and it can only be used for short periods of time because of over-loading of the collection surface.

Two sizes of Rotorods are available commercially from Metronics (Fig. 1). The U-shaped brass Rotorod has collection surfaces 1.59 mm in thickness, with arms 6 cm high, 8 cm apart and samples 120 liters per minute (lpm). It was designed to sample particles in the 15-25μ diameter range. The H-shaped chromel Rotorod is 0.38 mm in thickness, with arms 6 cm high, 12 cm apart and samples 41.3 liters per minute. It was designed to sample fluorescent particles (specific gravity 4.0 g cm^{-3}) in the 1-5μ diameter range (10). The Rotorod motors revolve at approximately 2400 rpm, with the collecting surfaces of the U- and H-shaped Rotorods revolving at 15.1 and 10.1 m sec^{-1}, respectively. The actual rpm for each motor varies and is supplied for each motor.

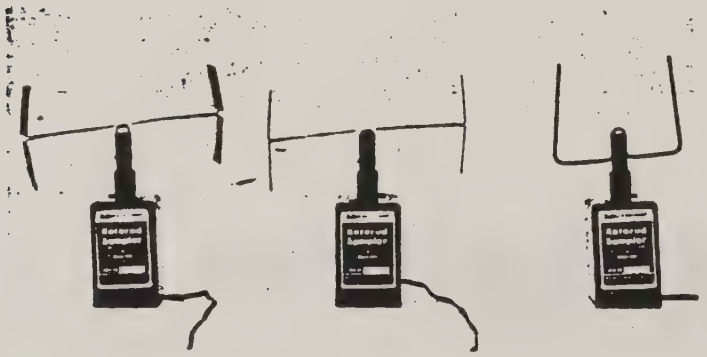

FIGURE 1. Rotorod samplers and motors. Commercially available Rotorods are the U-shaped (right) and H-shaped (center). Modified Rotorod is on the left.

DISCUSSION

Most researchers who are interested in collecting airborne spores usually wish to know the spore concentration in the atmosphere. Erroneous spore concentrations, however, can be calculated if collection efficiencies are not considered for the particular spore size under consideration. Not all spore sizes, even within the suggested range of the instrument, are collected with equal efficiency. Sampling sensitivity is low if efficiency is low, and low efficiencies also result in uneven distributions of spores on the collection surfaces (8). This is important to consider if sample fields are to be counted on the Rotorod arms.

Noll (8) has discussed theoretical and experimental aspects of whirling arm samplers. The collection efficiency (E) is largely a function of the particle parameter (P) (Fig. 2). Experimental data in this figure were obtained from a 16-stage rectangular collector impaction sampler developed by Noll. Each stage was designed to sample a specific size range of particles at 85-100% efficiency. The particular data used were derived from two stages designed to collect particles down to 26 and 13μ respectively. These stages had collection surfaces 3.2 and 0.8 mm in width, respectively, and revolved at 7.2 m sec^{-1}. Noll's data generally agree with experimental data of Chamberlain and Gregory for impaction of Lycopodium spores on cylinders (3). The efficiency suggested by Chamberlain for P equal to 10, however, is lower than those values suggested by Noll. Noll's data are preferred in practice because they are derived from a whirling arm sampler similar to the Rotorod, with similar sized rectangular collection surfaces, revolving at similar velocities. The line through the data was drawn as the line of best fit by eye.

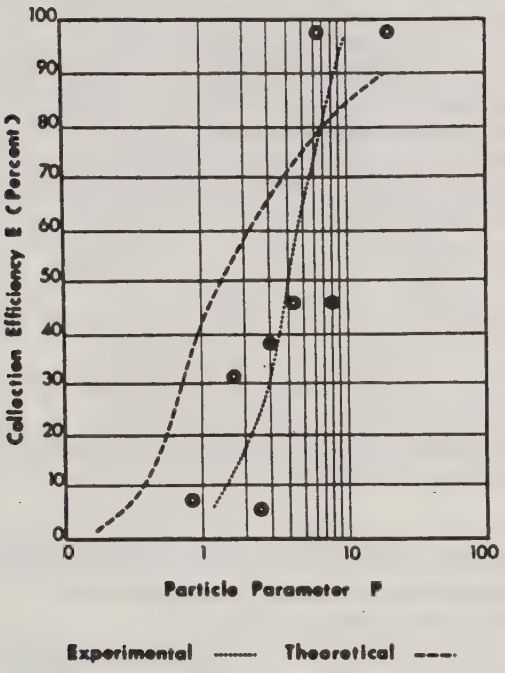

FIGURE 2. Relationship between particle parameter (P) and spore collection efficiency (E). (After (8)).

The theoretical curve presented in Figure 2 was determined by Langmuir and Blodgett for flow around a ribbon (8). Experimental values of E are lower than theoretically derived values, for P less than 7, but higher for P greater than 7. Chamberlain noted that experimental values of E were always lower than theoretical. He is uncertain whether this represents a fault in theory or a failure by sticky cylinders to retain all spores striking them. It would appear that the desired efficiency of 100% is approached as P approaches values of 10 or greater. In practice, because of the inconsistent agreement with theory, it is preferable to use the experimentally derived curve to determine E.

The selection of a suitable sticky material for the leading edge is important. If the surface is dry, particles bounce off. The material must be sticky, but if it is too thin, friction causes it to run off. If it is too thick, the edge loses its sharpness, the effective size of the collection surface is increased, and the collection efficiency is lowered. A 1:3 rubber cement and xylene solution used by Harrington, et al. (7), Froyd (5), and Edmonds (4) appears to give reasonable results.

The following is a general formula to calculate P for any spore size:

$$P = \frac{V_o \, d^2 \sigma_p}{18 \, \eta \, L \, S}$$

P = Particle Parameter (dimensionless)
V_o = Average* Rotorod arm velocity (cm sec^{-1} U-shaped (1010) H-shaped (1510)
d = Diameter of sphere of equivalent volume to that calculated for the spore (cm)
σ_p = Density of spore (g cm^{-3})
η = Viscosity of air (poises, g sec^{-1} cm^{-1}), at 18°C = 182.7 x 10^{-6} poises
L = Width of rectangular collector (cm)
S = Dynamic shape factor of particle (dimensionless)

*Actual arm velocity is variable because rpm vary from motor to motor.
Actual rpm for each motor is supplied by manufacturer.
The value of E is read from Figure 2.

Fuchs (6) has suggested that S is 1.28 for ellipsoids with ratio of axes, major/minor = 4. For practical purposes, no great error is made by setting S = 1 for spores with ratio of axes less than 4.

Table 1. Values of particle parameter (P) and collection efficiency (E) of U-shaped Rotorods in spore sampling studies.

Author	Organism	Type of spore[a]	Average dimensions of spore (μ)	Diameter of spherical spore of equivalent volume (μ)	P particle parameter (from formula)	E Percent (from Figure 2)
Asai (1)	Puccinia graminis	Uredospore	24 x 18.5	22	9.4	95
Froyd (5)	Hypoxylon pruinatum	Ascospore	26 x 10.5	19	7.0	80
Barksdale (2)	Piricularia oryzae	Conidia	23 x 8.5	16	4.9	65
Skilling (9)	Scleroderris lagerbergii	Ascospore	19.5 x 5	12	2.8	30

[a]Spore density was assumed to be 1.0 and S = 1 (ratio of axes of spores is less than 4). Diameter of equivalent sphere is rounded to nearest whole number.

Asai, Froyd, Barksdale, and Skilling used U-shaped Rotorods in their experiments. Asai, however, was the only investigator to mention sampling efficiency, noting that spores in the vicinity of 20μ diameter are impacted at approximately 100% efficiency.

Table 1 shows values of P and E for each of the four studies. The Puccinia graminis spores trapped by Asai are impacted at close to 100% efficiency. The other spores are sampled at much lower efficiencies. Unit densities for spores was assumed.

The H-shaped Rotorods will impact spores of unit density down to 9μ in diameter at close to 100% efficiency. Thus, in selection of Rotorod size to be used, it is important that sampling efficiencies for both sizes of Rotorods be determined for the particular spore in question, in order to obtain maximum efficiency. If P is greater than or equal to 10, the efficiencies close to 100% can be obtained.

H-shaped Rotorods can also be modified (Fig. 1) to a particular efficiency by welding aluminum shims of different widths to the arms, thereby adjusting the width of the collection surface and increasing the collection efficiency. Welding of shims to arms increases the aerodynamic drag on the rotating arms, which probably results in small decreases in the rotation speed of the sampler perhaps of 5% or so. This was not checked in practice, but it should be considered. This modification shown in Figure 1 was used by the author to collect spores of Fomes annosus (4.5-5.0μ diameter)(4) at an efficiency similar to that of fluorescent particles (specific gravity 4.0 g cm^{-3}, 3.0μ average diameter) collected on H-shaped Rotorods. This enabled a direct comparison of their respective dispersion patterns, with the object of determining if fluorescent particles could be used for tracing spore dispersal.

Another factor to be considered in collection efficiency is that of possible changes in shapes and, thus effective sizes of fungus spores while they are airborne due to changes in moisture content, and so forth. This is difficult to assess and thus has not been considered in the calculations.

CONCLUSION

In sampling fungus spores with a rotating arm impaction device such as the Rotorod, it is important to consider the collection efficiency for the particular species of fungus spore being sampled in order to make accurate calculations of the concentration in the atmosphere. If the particle parameter P is calculated to be greater than 10, then the efficiency of collection is close to 100%. If P is less than 10, then the collection efficiency can be read from Figure 2.

An alternative to this is to modify the size of the collecting surface to increase collection efficiency.

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Reprinted from *Crop Science*
Vol. 24, March-April 1984, p. 375-377

A QUANTITATIVE SAMPLING METHOD FOR AIRBORNE SWEET CORN POLLEN UNDER FIELD CONDITIONS[1]

P. K. FLOTTUM, D. C. ROBACKER, AND E. H. ERICKSON, JR.[2]

Abstract

The rate of pollen dehiscence in a sweet corn (*Zea mays* L.) plot was measured using a Rotorod Sampler®. Samples were taken from 0700 to 1230 h for 3 days during anthesis. Totals were averaged over the 3 days, and the resulting composite data were used to develop a sampling protocol accurate for determining the rate of pollen release. Results showed that a 10-min sampling period, with a frequency of at least once every half hour was required to accurately reflect the pollen release rate.

Additional index words: **Pollen, Dehiscence, Pollen emission profile, *Zea mays* L.**

STUDIES of pollen dehiscence in sweet corn (*Zea mays* L.), and many other grasses (Gramineae) have been primarily concerned with developing techniques that predict the date flowering will begin (Cross and Zuber, 1972; Gardner et al., 1981; Hanway 1966). In sweet corn the process of pollen dehiscence, usually defined only as anther decention, is fairly well understood (Knox, 1979; Percival, 1969). However, studies of patterns of pollen release for a single day or for the period of anthesis have generally been qualitative in nature, as quantitative in vivo measurements of airborne sweet corn pollen have not been made.

This paper 1) describes a method used to measure the pollen emission patterns in a flowering sweet corn field, 2) documents the effectiveness of the method, and 3) presents data pertinent to the optimal use of the method.

Materials and Methods

A Rotorod Sampler®[3], a rotating impaction device powered by a 12 v battery, was used to collect airborne sweet corn pollen. Airborne pollen is captured on the leading surface of removeable 64-mm plastic rods held by the rotating arms (Fig. 1). General Electric G-697 Silicone Grease®[3] facilitates capture and retention of the pollen grains. The arms are rotated at ca 2400 RPM, sampling a volume of ca. 120 L/min. Operating efficiency, or ability to collect airborne pollen in the volume sampled, was determined to be greater than 99% by manufacturers specifications.

These studies were conducted at Madison, Wis. during 1981. The Rotorod Sampler was positioned centrally in a 40 m^2 plot of 'Commander' sweet corn with the maximum height of the sampler rods slightly below center of representative tassels in the plot. Sample rods were replaced at the end of each sampling interval whereupon the number of captured pollen grains were counted.

Two concurrent collection procedures were designed and

Fig. 1. The Rotorod Sampler®, showing removable 64 mm plastic rods.

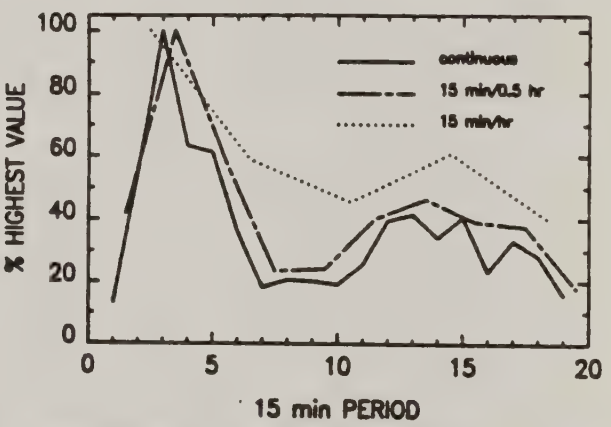

Fig. 2. Relationship of continuous, 1 sample/h and 1 sample/0.5 h profiles.

their results compared. The first procedure, subsequently referred to as the continuous method, consisted of running one Rotorod Sampler for 22 consecutive 15 min periods, from 0700 to 1230 h, for 3 days in the flowering sweet corn plot. Actual numbers of collected pollen grains were converted to pollen grains/L and plotted by period to develop a pollen emission profile for each day. The three daily profiles were then aligned so that the 15-min periods containing the initial daily peak coincided. This was done in order to accommodate the relative times of pollen dehiscence, not absolute time of day. Initial daily samples containing no pollen were not included in the analysis. Thus, the resulting composite profile consisted of 19, 15-min periods (Fig. 2).

To determine if reliable data could be obtained from fewer sampling periods than the 19 used to develop the composite of the continuous sampling method, the same data were reanalyzed assuming 1) one 15-min sample/h and; 2) one 15-min sample/0.5 h. For the one sample/h method, three groups of 15-min periods were analyzed as

[1] This research was supported in part by a grant from the Wisconsin Food Processors Assoc. Received 26 Apr. 1983

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[3] Ted Brown Assoc., 26338 Esperanza Dr., Los Altos Hills, CA 94022. Mention of a trade name does not constitute a guarantee or warranty of the product by the USDA nor an endorsement over other products not mentioned.

if each group represented a distinct replication of the method. From Fig. 2, nonrandomized groups used were:

Group 1: periods 1,5,9,13, and 17
Group 2: periods 2,6,10,14, and 18
Group 3: periods 3,7,11,15, and 19

For the one sample/0.5 h, two nonrandomized groups were analyzed as distinct replications:

Group 4: periods 1,3,5,7,9,11,13,15,17, and 19
Group 5: periods 2,4,6,8,10,12,14,16,18, and 19[4]

Regressions of pollen emission on period were conducted for each of the three data arrangements (continuous, one 15 min sample/h and one 15 min sample/0.5 h) using orthogonal polynomials to represent period numbers.

The second collection procedure used consisted of running another Rotorod Sampler® for a period of only 10 min/sample. Samples were collected for the first 10 min of periods 1,3,5,7,9,11,13,15,17, and 19, (one 10 min sample/0.5 h), with the sampler left idle for the remaining 5 min of the period. These samples were neither randomized nor replicated. Results of these 10-min samples were analyzed as above and compared to the composite results and to the results of the one 15-min sample/0.5 h.

Results and Discussion

Method 1

Daily pollen emission profiles were strikingly similar. A large peak was recorded during one of the first 3 15-min periods each day. This was followed by a reduction in emission intensity, another slight increase then decreasing thereafter.

Regression analyses to determine the relationship between the amount of pollen collected and the time of day demonstrated significant linear ($P < 0.05$), quartic ($P < 0.01$), and quintic ($P < 0.05$) coefficients for each of the three daily profiles and the composite.

Regression coefficients for the one sample/0.5 h method, and for the composite profile (continuous method) were not significantly different (linear = -0.01 vs. -0.01; quartic = -0.004 vs. -0.002; quintic = 0.005 vs. 0.001, respectively - regression coefficients not converted from orthogonal polynomials). Similar analyses for the one sample/h method showed linear significance ($P < 0.05$), but neither the quartic nor the quintic coefficients were significant. Therefore, by inspection and analyses interpretation, it is shown that the curves for the continuous and one sample/0.5 h data arrangements are clearly high degree polynomials, while the curve for the one sample/h data arrangement is only a first degree relationship. These relationships are represented graphically in Fig. 2. Had sampling begun earlier each day, the initial peak may have been evident in the one sample/h arrangement, but this would not alter the lack of significance for the second peak.

Method 2

Results of the second technique, running the sampler for a period of 10 min/0.5 h, were compared to the results of the continuous method and to those of the one 15 min sample/0.5 h data arrangement. These were not significantly different at the levels previously noted as there were no differences in the rate of pollen collection or shapes of the profile curves. Hence, one 10 min sample/0.5 h retained the accuracy of continuous sampling and displayed the bimodal emission profile of the sweet corn population. Further, this sampling frequency optimized accuracy of the data and reduced the effort required in data acquisition. Moreover, it permits one operator to gather data simultaneously from several locations in a large field.

Pollen release is dependent on several environmental variables (Flottum et al., 1983), as is the amount of pollen collected on the sampler. For this reason, use of the sampler cannot accurately determine when anther decention and subsequent pore formation occurs, but rather when the released pollen becomes airborne. In spite of this, the Rotorod Sampler®, when used in the manner described, is an accurate method of recording the pollen emission patterns in a sweet corn field.

[4] Nineteen used again for balance.

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STEDP-MT-L-A(ROME) 25 SEPTEMBER 1991

MEMORANDUM FOR U.S.D.A. FOREST SERVICE (JACK BERRY)

SUBJECT: SOP FOR ASSAY OPERATIONS /MICROBIOLOGY LABORATORY

1. ENCLOSED IS THE SOP WE DISCUSSED. THIS SOP ALSO HAS THE FORMULA FOR THE PLATE MEDIUM THAT WE USED WITH THE BT ON PAGE 9.

2. THANKS FOR THE WORK. WE ARE LOOKING FORWARD TO DOING THIS AGAIN NEXT FALL.

WILLARD ROME

LIFE SCIENCES DIVISION/BIO ASSAY BRANCH 20 OCT 1987

ASSAY PROCEDURES FOR BIOLOGICAL SIMULANTS/~~PREPARATION AND SAMPLER~~ Sampler Preparation

DTC SOP 70-100

CHIEF, ASSAY BRANCH

I. RESPONSIBILITIES:

A. Will provide training and supervision for personnel assigned to various laboratory duties.

B. Will assure compliance with this SOP.

MICROBIOLOGIST IN CHARGE

II. RESPONSIBILITIES:

A. Will understand and implement the procedures outlined in this SOP.

B. Will be responsible for producing simulant in quantities needed to support field and laboratory testing, and for quality control of simulant in accordance with test plan criteria.

C. Will maintain continuous surveillance of simulant storage conditions and techniques, and field and laboratory sampling procedures to verify they are consistent with sound quality control and microbiological procedures.

MICROBIOLOGIST AND TECHNICIANS

III. LABORATORY PROCEDURES:

A. PREPARATION AND ASSESSMENT OF THE WAGNER SAMPLER.

1. Preparation of the Wagner Sampler: The vacuum stem and sampling stem of clean Wagner samplers will be plugged with non-absorbent cotton, sterilized and dried in a sterilizer. Samplers are then removed and

cooled. In a clean area, the samplers are taken apart and sterile filters are inserted over the sampler mat using a pair of sterile forceps. The forceps are "flamed off" at regular intervals to prevent contamination. The samplers are reassembled with identification tags attached and the appropriate number of samplers for each station placed in a carrier. The carrier is identified as to crew and station. The samplers are then ready for storage or for distribution.

2. Assay of the Wagner Sampler: After the Wagner samplers have been returned from the field, the sampler will be broken down and, with the aid of sterile forceps, the filter and the mat removed and placed into sterile 50ml screwcap tubes contianing 10ml of gelatin phosphate diluent. The tube is then identified as to the station number and sampler sequence. The forceps are flamed between samples to prevent cross contamination. The samples are then shaken for 10 minutes on a mechanical shaker to suspend the BG prior to assay.

3. Preparation for Assay (i.e. plating): The appropriate number of casitone media plates are removed from refrigerated storage and set out at room temperature before plating begins in order to allow any condensate to dry off. An adequate supply of the following equipment is required:

(a) 9ml dilution blanks with the appropriate diluting fluid.
(b) Sterile 1.0ml pipettes graduated in 0.01ml.
(c) Sterile spreaders
(d) Pipette and spreader discard pans
(e) Felt tip marking pens

A disinfectant (i.e. 70% alcohol) will be kept available for disinfecting spilled materials and surfaces.

4. Assay Technique: The plating crew will consist of two persons. One person will act

as the "spreader" and will be responsible for marking plates with the necessary identification and spreading the inoculum on the plates; the other person will act as the pipettor and is responsible for making dilutions and placing the inoculum on the plates.

(a) The "spreader" first checks the plates for contamination and then numbers the plates with the sample number and dilution. Duplicate plates are made on each dilution. Undilute plates are marked with a "U". Consecutive dilutions are numbered 1,2,3, etc. The plates are then passed to the pipettor.

(b) The pipettor mixes the undiluted sample by using a laboratory test tube vortex mixer swirlling the liquid up the tube ten times. He then uses a 1.0ml pipette and draws up 1.0ml of fluid, then touches the tip of the pipette to the inner wall of the tube to remove any excess fluid. While holding the pipette at an angle of about 30 degrees, and with the tip of the pipette resting on the agar surface, 0.2ml is delivered to the plate marked "U". When the inoculum is delivered, the pipette is touched on a dry portion of the agar and drawn through a one-inch arch to remove fluid adhering to the tip. This procedure is repeated on the duplicate plate.

(c) The plates marked "U" are then passed back to the "spreader" to be spread.

(d) Using the same pipette, the pipettor again draws 1.0ml of the fluid from the undiluted sample. The contents of the pipette are then delivered to a 9ml dilution blank. The pipette is then placed into the pipette discard pan. The tube is mixed as before. Using a clean sterile pipette, the fluid is drawn and plated using the same technique as the undiluted sample. This plate will be the U+1 dilution (i.e. 1:10). This procedure is repeated for all subsequent dilutions required, changing pipettes between each dilution before mixing. The number of dilutions and the dilutions to be plated are

determined beforehand. When a higher concentration of organisms is suspected, more dilutions are made, and the lower dilutions are not usually plated in order to conserve time and material.

(e) After the inoculum has been delivered on a plate, they are passed to the "spreader". The "spreader" takes a clean, sterile spreader and spreads the fluid by using a circular motion, starting in the center of the plate and working toward the edge. Care is taken to keep the fluid from being thrown upon the sides of the plate, and a straight movement across the plate may be necessary for uniform distribution of the inoculum over the entire surface of the agar. The spreader is kept in contact with the agar surface at all times. Agar plates containing bubbles or contaminating growth or too thin to support growth are discarded before plating. Used spreaders are placed into the pipette discard pan.

(f) All undilute samples and dilutions are stored at $4^{o}C$ until data are obtained for each sampling station. (Re-assay may be required in cases of missed dilutions or challenged estimates.) If an accident should occur resulting in the spillage of sample, the contaminated area is flooded with 70% alcohol and wiped up to prevent contmination of other equipment.

(g) Immediately after the assay of the samples is completed, the identification tags or tapes are removed from the tubes and samplers before sterilizing and all used glassware and equipment or that which has come in contact with potentially contaminated material is sterilized. The plating area is then washed down with 70% alcohol.

(h) The spread plates are placed in a pan by the "spreader" right side up, one sample per stack, with the undilute plates on the bottom and the highest dilution on top, and the inoculum allowed to dry. When the inoculum has soaked into the medium on the last plate spread, the plates are then inverted and placed on the incubator shelves.

Plates are incubated at 37°C and require 18 to 24 hours to develop countable colonies.

5. Counting Plates: All plates that have 300 or less colonies are counted except in cases where the highest dilution has more than 300 colonies or when the plates are not countable due to contamination or plating error. In these cases, an estimate of the count may have to be made on the next countable dilution. If it is not feasible to count all of the colonies, the plate is divided into sections, by aid of guidelines on the counter, and one or more sections are counted and the count multiplied by the correct factor in order to obtain an estimate of the total count. After plates have been counted, they are sterilized, Prior to sterilization the plates are placed in pans and covered with water. A small quantity of detergent is added to the water. After sterilization, the melted agar is washed down the drain and the melted plates are placed in plastic garbage bags and disposed of in a dumpster.

B. PREPARATION AND ASSAY OF THE REYNIER SLIT SAMPLER: The Reynier sampler uses a clock, which may be either AC or DC current, to sample air containing a biological aerosol over a period of time from 1 minute to 2 hours depending on the Reynier sampler used.

1. Preparation of the Reynier Slip Sampler:
(a) Slit Adjustment: The width of the slit opening will be set at 0.006 inch when using the sampler at a sampling rate of 1 cubic foot per minute. This is done by loosening one of the screws in the slit assembly and inserting a 0.006 inch leaf type feeler guage. By holding the slit assembly tight against the feeler guage and tightening the screw, the slit is adjusted for the correct clearance.
(b) Cleaning Slits: Slits are cleaned with a one-half inch brush to remove all debris in the slit area. A clean brush is

then dipped in 70% alcohol and the complete slit area, including the undersurface, brushed off and dried.

(c) Timer adjustment: The timer is set by rotating the petri plate retainer clockwise until the pointer is on or past the zero mark. Turn the clock release on for a few seconds to insure proper running condition of the clock mechanism.

(d) Plate Installation: The appropriate plates containing agar are used. Remove the cover of the Reynier sampler exposing the plate retainer. Mark a starting line on the bottom of the petri plate with a permanent marker and install the plate on the retainer, lining up the mark on the plate with the zero mark on the clock. Replace and tighten cover.

(e) Slit Height Adjustment: Adjust the slit height by releasing and lowering the slit height guage until it rests lightly on the surface of the agar. Adjust the slit-to-agar surface distance by turning the slit tube knob until the pointer on the height guage corresponds to the 4mm mark on the tube scale. Raise and tighten the height guage with a counter-clockwise movement. Check to insure that the sampler identification tag is securely on the sampler.

2. Assay:

(a) At the conclusion of the sampling period the samplers will be returned to the laboratory and the plates removed after the external surfaces of the sampler have been wiped with 70% alcohol. The plate lids are replaced with the appropriate identification written on the lid. All plates are incubated at 37^{o}C for 18-24 hours. After incubation, the plates are counted using the 12 or 6 degree segmented grid for the 2 hour Reynier clocks. Each segment corresponds to four or two minutes of sampling time respectively. The plates will be positioned so that the starting mark made on the plate will correspond to the "start" line on the

counting grid.

(b) The number of colonies per segment will be counted. All colonies on the lines defining the left margin of each segment will be counted as belonging to the segment. If the segment contains too many colonies for an accurate count it will be marked "TNTC" (too numerous to count).

C. PREPARATION AND ASSAY OF THE ALL-GLASS IMPINGER (AGI) AND PRE-IMPINGER:

1. All-Glass Impinger:

(a) All inpingers will be calibrated at a flow rate of either 6.0 or 12.5 liters (l) per minute, as specified by the test operations plan.

(b) The impingers will be washed and thoroughly rinsed in distilled water. The clean, dry impinger tops and bottoms will be assembled, and the inlet and outlet tubes plugged with non-absorbent cotton and sterilized.

(c) The sterile impingers will be filled aseptically with the appropriate, sterile collecting fluid. Six l/minute impingers will have 18.5 ml of fluid. 12.5 l/minute impingers will have 20.0 ml of fluid.

(d) After filling the impingers, the tops will be secured to the bottoms by placing a rubber band around the neck at the bottom and slipping it over the outlet stem of the impinger top. Labels will be placed on the impinger for station identification.

(e) The impingers will be arranged in racks according to crew listing and will be numbered according to station number.

2. Pre-Impingers:

Dry, plastic pre-impingers usually will not be assayed. Each pre-impinger will have a piece of rubber tubing which is rigid enough to prevent the pre-impinger from sagging or drooping when connected to the impinger.

3. Assay of AGI Samples:

(a) Measuring volume of collecting fluid:

To calculate the number of colony forming units (CFU) collected, the volume of the fluid remaining in the impinger after operation must be determined. The contents of the impinger will be poured into graduated test cylinders and readings taken to the nearest 0.1ml. The adhesive labels bearing the number of the impinger will be transferred to the tube to label the sample. The volume of the impingers will be recorded.

(b) Plating will be done using the plating technique for the assay of the samplers.

D. PREPARE AND ASSAY OF THE 6-STAGE ANDERSEN SAMPLER:

1. The samplers should be inspected and free from dust and dirt. The holes in the 4th, 5th and 6th stages should be examined under a stereoscope (10X). Any plugged holes should be carefully punched out with a cleaning wire of appropriate diameter. When working with simulants, it is not necessary to sterilize the samplers. However, a 70% alcohol solution should be used to wipe the samplers between tests.

2. The Andersen plates (glass) will be brought to ambient temperature before using. The samplers will be loaded in a sanitary area by personnel dressed in clean clothes. Always start loading the sampler with six plates in a stack - numbered from six (bottom) to one (top). Putting hard pressure on the top stage of the Andersen, release the 3 spring fasteners. Remove the six stages of the sampler and begin loading by placing the No. 6 dish on the base and the No. 6 stage over it. The remaining plates and stages will be assembled in descending order. Putting hard pressure again on the top stage of the Andersen, hook the sampler fasteners, taking care to insure that the stage gaskets are in place. The sampler is then stoppered and marked for identification.

3. The Samplers will be placed in

containers and the containers identified with the appropriate station number.

4. After exposure, the samplers are collected and returned to the laboratory. The exterior of the sampler container is wiped with 70% alcohol and the sampler plates removed. The lists are replaced on the plate bottoms, the plates identified as to stage and sampler number, and incubated at 37°C.

5. After incubation (18-24 hours) the plates are counted. Data will be reported to the microbiologist in charge. The microbiologist will use the appropriate form to report the data.

E. PREPARATION OF DILUENT/COLLECTING FLUID: Gelatin phosphate is generally used as a diluent and collecting fluid for BG and prepared as follows:

NA_2PO_4..... 4.0g/1
Gelatin..... 2.0g/1
Deionized H_2O... 1 liter

Dissolve the ingredients in water by heating. Adjust the pH to 7.0 ± 0.1 with 5N HCl. Sterilize in an autoclave 15 psi for 20 minutes or longer depending on the size of the container and quantity being sterilized. Add Antifoam A (1ml of a 1:10 dilution per 1) to suppress foaming.

F. PLATES:

1. Preparation of Plating Medium: The plating medium for BG is generally Tryptose Agar and is prepared as follows:

Bacto-Casitone..... 20 gms/1
Dextrose..... 10 gms/1
Agar Agar..... 20 gms/1
Sodium chloride..... 5 gms/1
Green food coloring. 0.3 ml/1

Suspend the ingredients in cool water and

stir while heating to a boil. After all ingredients are dissolved, adjust the pH to 6.9 ± 0.1 with 5N NaOH. Add actidione (10 ml of a 1% aqueous solution) for each liter of plating medium. At temperatures below freezing tryptose agar plating media is prepared as follows for Andersen or Reynier samplers:

Bacto-Tryptose.....	20 gms/l
Dextrose.....	10 gms/l
Agar Agar.....	15 gms/l
Sodium chloride.....	5 gms/l
Green food coloring....	0.3 ml/l
Methyl cellulose (CMC).	25 gms/l

G. PREPARING PLATES:

1. Regular AGI and Wagner Sampler Plates: The media is prepared as above and cooled to the appropriate temperature. 20 mls of medium is dispensed aseptically into each sterile petri dish by using a calibrated Brewer pipetting machine. Plates are poured on a flat level surface that has been washed down with a solution of sodium or calcium hypochlorate (66 gms/gallon) and wiped dry before the plates are set out. If necessary, the plates may be stacked 5 or 6 high and poured from the bottom up to conserve space. After plates are poured and the medium has solidified, they will be inverted and allowed to dry and age for a minimum of 24 hours. Aged plates will be stored in a 4 degree C refrigerator until used.

2. Andersen Sampler Plates: Glass Andersen Sampler plates are poured using the same procedures outlined above except that 27 mls of medium is dispensed into each sterile glass Andersen Sampler plate.

3. Reynier Sampler Plates: Reynier Sampler plates are poured using the same general procedure as above except that 80 mls of liquid medium is dispensed

into each sterile plate and plates should never be stacked until the medium has solidified.

H. REPORTING DATA:

1. Data will be reported on the appropriate MT-L-A forms provided for that purpose.

2. The microbiologist in charge will review the data, explain inconsistencies if possible, and make duplicate copies, one copy for MT-L-A and one copy for the Project Officer.

Nozzle	BEECOMIST 360A	Slice Rate	1.5 MHz
RPM	12700	AVG	20000
Spray Pressure	30 psi	DFM	1 cm.
Airspeed	50 mph	BAR	1.5
Flow Rate	.47 gpm	Distance to Probe	25 cm.
Tank Mix	THURICIDE 48LV,NEAT	Sample Interval	60 sec.
		Number of Samples	1
FILE: C:\PMS\DATA\08288511.003		Number of Sample Rings	9

Number of Tests Combined:2

UPPER LIMIT	N(RAW)	N/SEC	Gm/SEC	% N	% VOL.	ACCUMULATED % N	ACCUMULATED % VOL.
56	20839	1.98E+08	6.50	82.14	26.09	82.14	26.09
89	21130	3.05E+07	6.06	12.66	24.30	94.81	50.39
122	18098	8.23E+06	5.00	3.42	20.05	98.23	70.44
154	13659	3.26E+06	4.46	1.36	17.91	99.58	88.35
187	6060	841250	2.18	0.35	8.74	99.93	97.09
220	1584	150823	0.66	0.06	2.65	100.00	99.74
252	90	8250	0.06	0.00	0.23	100.00	99.96
284	6	932	0.01	0.00	0.04	100.00	100.00
TOTAL	8.15E+04	2.41E+08	24.92				

TOTAL ACCEPTED RAW PARTICLES / TOTAL IMAGES = 81466/ 103182 = 79.0%

NUMBER MEAN DIA.= D_{10}.... 48.04 µm
VOLUME MEAN DIA.= D_{30}.... 58.29 µm
SAUTER MEAN DIA.= D_{32}.... 72.59 µm

NUMBER MEDIAN DIA.= $D_{N.1}$... <56 µm
$D_{N.5}$... <56 µm
$D_{N.9}$... 76.74 µm

VOLUME MEDIAN DIA.= $D_{V.1}$... <56 µm
$D_{V.5}$... 88.74 µm
$D_{V.9}$... 160.52 µm

RELATIVE SPAN= 1.42 Extrapolated

Reference #8

FIELD CREW CHECK SHEETS AND REPORT

CREW: _____

TRIAL: _____

DATE: _____

A. Inventory	Qty Required	Qty Picked-up	Remarks
Samplers			
Rotorod			
Rotorod holder			
Rotorod motor			
Mylar w/holder			
Reynier			
Equipment			
Vacuum pump			
Tubing			
Stakes			
Generator			
Fuel			

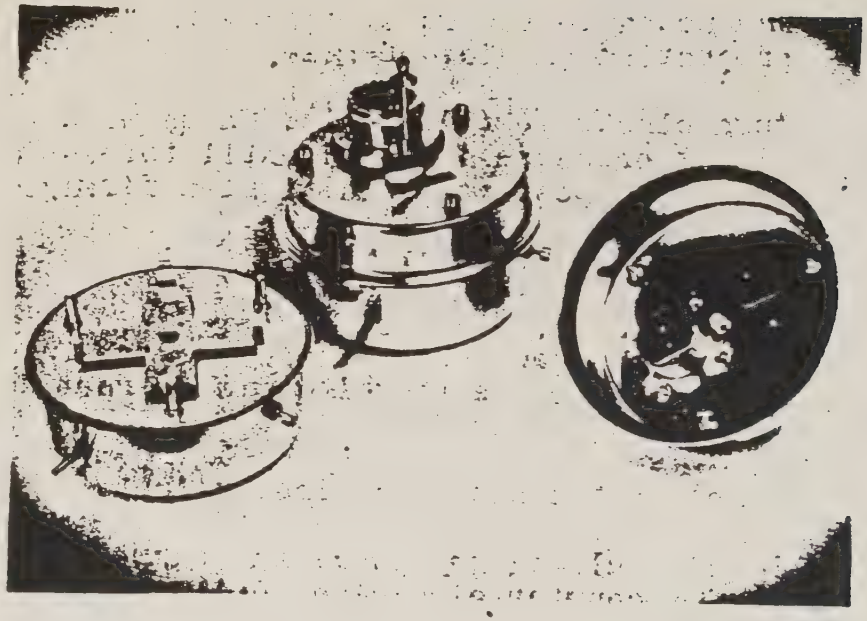
Note: Suggest this form be used by each crew leader to insure that all items are picked-up for the field trial.

REYNIERS & SON

3806 N. ASHLAND AVENUE

CHICAGO 13, ILL.

GRaceland 2-0015



REYNIERS SLIT SAMPLER

Model #FD-100

Complete with an adjustable slit tube threaded into the top of the sampler and provided with a height indicator. The slit is adjustable from .004 minimum to .125 maximum. It accommodates a 150 mm x 20 mm culture plate. It is available in a 30 minute, a 60 minute, or 120 minute timing mechanism. All timing mechanisms are interchangeable.

DIRECTIONS

1) To remove top of a slit sampler, turn (3) knurled knobs counter clockwise with a slight pull upwards and continue to unscrew until they stop.

2) Push timer stop in.

3) Set timer to desired time.

4) When replacing sampler top make sure the notch in top is aligned with the projection on side of base.

5) Turn clockwise (3) knurled knobs with slight pressure downward until threads engage in post and then tighten firmly.

6) Pull timer stop out.

7) To set gauge:
   a) Unscrew gauge with clockwise motion letting it come to rest on top of dish.

   b) Set scale to desired height.

   c) Pull gauge upward and turn counter clockwise until sealed against "O" ring.

8) To set width of slit in sampling tube: (Slit is adjusted to 0.152 mm when shipped, approximately .006 of an inch.

   a) Loosen (2) screws.

   b) Set to desired opening.

   c) Tighten.

9) To change timer plate assemblies:

   a) Unscrew knurled thumb rings on posts.

   b) Pull timer stop all the way out.

   c) Pick up timer plate assembly.

   d) Place desired timer plate assembly on posts, making sure the hole at the 15 minute mark (on the 30 minute timer) is placed on the post between the timer stop and the vacuum hose connection. On the 60 minute plate the hole at the 30 minute mark and on the 120 minute plate the hole at the 60 minute mark should be placed as stated above.

   e) Replace knurled thumb rings on post.

PROCEDURE PERTAINING TO THE USE OF THE MECHANICAL INTERVAL TIMER SLIT SAMPLER

1. The width of the slit opening should be set at 0.006 inch (approximately .152 mm) when using the sampler at a sampling rate of 1 cfm, resulting in an impingement velocity on the media of approximately 220 F.P.S. The use of a feeler gauge provides an accurate means of measuring this width.

2. Prior to the use of the slit sampler, wind clockwise, the mechanical timer. The timer stop on the side of the sampler should then be pushed in to prevent operation of the clock until actual sampling time.

3. The slit opening does not serve as a critical orifice. A flowmeter or a calibrated "U" tube water manometer is to be used in conjunction with the sampler, and located between the sampler and the vacuum source. If a calibrated "U" tube manometer is to be used, the calibration for 1 cfm should be determined by connecting a rotometer to the slit sampler by means of an adapter and placing the water manometer between the slit sampler and the vacuum source.

4. Inspect and clean the slit opening prior to each use, to remove dust and dirt which may have accumulated from previous tests.

5. Place a petri dish, containing the appropriate agar, in the slit sampler and make a mark on the dish to indicate its position under the slit at the start of the sampling. If necessary, slight adjustments of dish holder can be made by gently bending clamps. Adjust the height of the slit above the

agar by use of the height indicator. Unscrew the height indicator and allow it to rest on the surface of the agar. Adjust the tube by means of the raising and lowering screw until the top of the height gauge is at the desired relation (2-3 mm) to the millimeter scale on the tube, which results in the proper distance between the slit opening and the surface of the agar. After this setting is made, lift height indicator until it engages threads on bottom of tube support, screw height indicator with counter clockwise rotation until seal with gasket is made to eliminate leakage.

6. When the sampler is ready for use, the vacuum is adjusted to operate at 1 cfm. To start timing mechanism pull timer stop OUT.

NOTE: When replacing or substituting other timing mechanisms (30, 60 or 120 minute) this timer release must be pulled completely back to its stop. When replacing the above mentioned timers the hole in the mechanism protector cover MUST line up with the stop shaft.

For further description of sampling devices and methods you may refer to:

Public Health Monograph No. 60
Sampling
Microbiological
Aerosols

Single copies may be obtained from:

Public Inquiries Board
U.S. Public Health Service
Washington 25, D.C.

Ted Brown Associates

Product Bulletin No. 1-77

AIR SAMPLING EQUIPMENT

Introducing...

Figure 1

The Rotorod® Sampler

Many people concerned with the type, size or amount of particulate material in the air are making use of a novel air sampling device called the ROTOROD Sampler. It is novel because instead of pulling air through a membrane filter with a vacuum pump, as most samplers do, the ROTOROD Sampler rotates precision made rods through the air at high speed and collects particles by impaction on the leading edges of the rods at sampling rates up to 120 liters/minute.

Figure 1 illustrates the basic ROTOROD sampler without collector rods in place. The ROTOROD consists of a constant speed 12 volt dc motor mounted in a protective case and equipped with a hub which accepts several types of collector rods. Overall dimensions, without rods, are 1½" x 1½" x 4". Two pairs of slots spaced 90° apart on the hub match the forked coupling on the collector rod which fits snug into the slots, and are held tightly on the hub. Figure 2 illustrates typical field mounting of the ROTOROD Sampler including battery with sufficient capacity to power sampler for more than 50 hours.

Figure 2

The "H" and "U" shaped collector rods shown in Figure 3 form the basic collector rod geometry. Since a narrow surface is more effective in collecting small particles while a wider surface is more effective for larger particles, Metronics has developed the 0.48 mm wide "H" shaped rod for collection of particles in the 1 to 10 micron range and the 1.59 mm wide "U" shaped rod for collection of particles in the 10 to 100 micron range.

ROTOROD Sampler turns at a nominal 2400 RPM which moves the collecting surfaces through the air and thus causes most of the particles within the air intercepted by the collector rods to become impacted on the leading flat-surfaced edges of the rods. To ensure that these particles are retained for later examination, the rods are coated with one of a number of tacky materials especially developed and tested for this purpose. The specific material selected is determined by the kind of particles to be collected and if they are to be examined under visible or ultra-violet light or are to be removed and cultured, e.g., airborne bacteria.

The shape of the particle collectors are incidental to their operation and are merely convenient shapes into which the rod materials are formed to provide adequate support and rigidity during the rapid spinning. The decided in-bend on all verticle arms of the "H" and "U" shaped collectors compensates for the centrifugal out-bending during spinning and causes the vertical collecting segments to sweep out a cylindrical annulus. This permits the ROTOROD to be accurately calibrated in terms of **the sampling rate in liters per minute.**

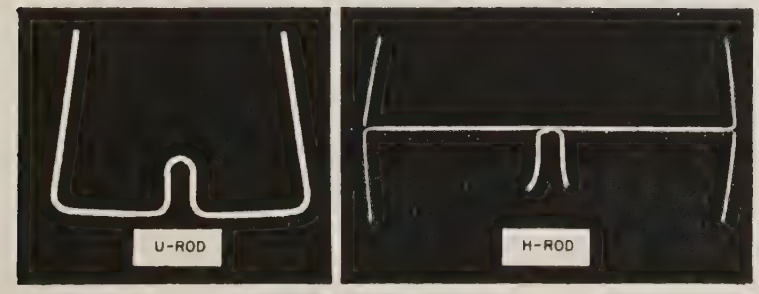


Figure 3

The "H" and "U" shaped rods are both formed from metal, chromel and brass respectively. The flat-surfaced leading edges are examined for collected particles using incident illumination. This technique works very well for the 1 – 5 micron fluorescent particles, which are frequently used in air tracer studies, where the particles are assayed using UV illumination. It also works very well for many types of naturally occurring dust and pollen using incident visible light. However, some materials require use of transmitted light which precludes the

OVER

use of the solid metal collectors. For this purpose a modified "U" shaped rod, called a "Fixed Collector Sampling Head" has been designed to use removeable 1/16" square, flat-surfaced clear plastic rods as the collecting surfaces. A pair of these straight "I" shaped plastic rods are coated with a tacky material, inserted in the holder for sampling and when the sampling is complete the clear-plastic I-rods are removed and mounted on a glass slide for microscopic examination.

In response to the needs of plant pathologists and other users of the ROTOROD sampling technique, an improvement was made so as to obtain a series of intermittent samples on a single pair of rods with extended periods of "off" time in-between sampling periods. Since unprotected tacky collector rods will continue collecting a few particles from natural air movement past the stationary exposed collector, a protective housing was developed into which the collectors automatically retract when the sampler is turned off. This device is called the "Retracting Collector Sampling Head" and like all other types of collectors, quickly slips into place on a basic ROTOROD Sampler hub. The collecting surfaces for this device are made up of two half-length Type-I rods which slip into place in the same manner as the full-length Type-I rods slip into the Fixed Collector Sampling Head.

The Retracting Collector Head device has been used to obtain pollen counts at pre-selected times each day, for averaging over a period of several days. Each sampler turns on at its same pre-assigned time each day and operates for a pre-set number of minutes. During the balance of the time the collectors are retracted. At each successive turn-on the collectors again pop out into sampling position by centrifugal force and remain so positioned as long as the sampler is in the "on" phase of the sampling cycle.

All items described above are listed on the ROTOROD Sampler Price List PL 1-77, plus accessory equipment and materials. For more information write or phone Ted Brown Associates. Listed below are additional ROTOROD information bulletins that are available on request.

PB No. 14-69 Pollen and Spore Sampling Equipment
PF No. 4-68 ROTOROD Bacterial Sampler – Viable Organism Recovery Indoors
PF No. 5-68 ROTOROD Bacterial Sampler – Viable Organism Recovery Outdoors

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